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FACULTAD DE CIENCIAS BIOLÓGICAS

DEPARTAMENTO DE ZOOLOGÍA Y ANTROPOLOGÍA FÍSICA

TESIS DOCTORAL

Advances in the systematics and phylogeny of allomalorhagid kinorhynchs (*Kynorhyncha, Allomalorhagida)*

Avances en la sistemática y filogenia de los kinorrincos alomalorrágidos

MEMORIA PARA OPTAR AL GRADO DE DOCTORA
PRESENTADA POR

Nuria Sánchez Santos

Director

Fernando Pardos Martínez

Madrid, 2015

Advances in the Systematics and Phylogeny of allomalorhagid kinorhynchs (Kinorhyncha, Allomalorhagida)

Avances en la Sistemática y filogenia de los kinorrincos alomalorrágidos (Kinorhyncha, Allomalorhagida)

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Tesis Doctoral 2015

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Memoria para la obtención al grado de Doctor en Biología presentada por

Nuria Sánchez Santos

Dirigida por Fernando Pardos Martínez

Madrid, 2015

FERNANDO PARDOS MARTINES, PROFESOR TITULAR DEL DEPARTAMENTO DE ZOOLOGÍA Y ANTROPOLOGÍA FÍSICA DE LA FACULTAD DE CIENCIAS BIOLÓGICAS DE LA UNIVERSIDAD COMPLUTENSE DE MADRID, CERTIFICA:

Que la presente memoria titulada “Avances en la Sistemática y la filogenia de los kinorrincos alomalorrágidos (Kinorhyncha, Allomalorhagida)” presentada por Dña. Nuria Sánchez Santos para optar al Título de Doctora en Biología, ha sido realizada en el Departamento de Zoología y Antropología Física de la Facultad de Ciencias Biológicas de la Universidad Complutense de Madrid bajo mi dirección. Y considerando que se presenta trabajo de Tesis, autorizo su presentación a la Junta de Facultad.

Y para que así conste, firmo el presente en Madrid, Junio 2015

La doctoranda

Nuria Sánchez Santos

V°B° del director

Fernando Pardos Martínez

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ABSTRACT

Basic concepts

Kinorhyncha is an exclusively meiobenthic phylum of free-living marine ecdysozoan invertebrates, comprising around two hundred described species. Kinorhynchs or mud dragons have small size, from 0.13 to 1.04 mm, and inhabit the interstices between grains of muddy to coarse sand sediments, mostly in the first, well oxygenated centimeters, from the intertidal zone to the deep sea bottoms all around the world. Ocassionaly kinorhynchs have been found on algae or associated to a variety of sessile invertebrates.

Currently, Kinorhyncha is included into the monophyletic Scalidophora, together with Priapulida and Loricifera. Scalidopohra and Nematoida (Nematoda plus Nematomorpha) are grouped as the Cycloneuralia. The group is well defined by morphological apomorphies, as the presence of a collar-shaped brain surrounding the pharynx, but its monophyly is hardly ever recovered in molecular analyses. Currently, Cycloneuralia is related to the Panartropoda (Tardigrada, Onychophora, and Arthropoda), both constituting the monophyletic Ecdisozoa. The Ecdisozoa is supported by molecular data and morphological characters such as the presence of non-ciliated cuticle that sheds periodically through apparently ecdysteroid hormones control.

All kinorhynchs present similar external morphology, with an elongated body covered by cuticle and divided into three regions: head, neck and trunk. The head is composed of an eversible introvert and a protrusible mouth cone. The neck consists of a ring of rigid plates, the placids, which operate as closing system when the introvert and the mouth cone are completely retracted into the body trunk. The trunk is formed by eleven segments, which may be either divided into cuticular plates or forming a closed ring. Furthermore, each segment is equipped with several external cuticular structures whose type, number and arrangement are traditionally used as taxonomic characters for Kinorhyncha. The head and neck are not considered as segmentary units but the segmentation is reflected in the trunk, both in the internal (nervous and muscular systems and epidermal glands) and external anatomy (plates, spines, setae, tubes, sensory spots).

The classification was established mainly based on the external morphology and the arrangement of segmental plates, especially those of segments 1 and 2, a feature directly related to the trunk closing system. However and even though the traditional classifications were founded on a very comprehensive knowledge of kinorhynch morphology, they do not reflect the evolution of the Kinorhyncha, but were absolutely phenetic and do not follow the Hennigian thinking. Therefore the phylogenetic relationships within the phylum have been mistakenly influenced from its taxonomical

classification; when actually, the conceptual process should run in the opposite way, that is, a classification should be inferred from a phylogenetic tree.

Founded on the segment organization, and the general external morphology, kinorhynchs have been traditionally distributed into two large orders: Homalorhagida and Cyclorhagida, with Homalorhagida consisting on a single suborder (Homalorhagae) and Cyclorhagida divided into three suborders according to the closing system. These are the Conchorhagae (closing bilaterally, shell-like), Critptorhagae (radially closing system, without placids) and Cyclorhagae (radially closing system, with placids).

This general classification has been more or less widely accepted, with new described taxa being fit into it until the advances of molecular phylogeny came up with surprising news. The results of these studies were mostly congruent with some groupings from the traditional classification and clarify most of the relationships amongst the major clades within Kinorhyncha. However, they also pointed out surprising clades that had not ever been proposed. The most relevant difference was the polyphyly of the traditional Cyclorhagida. Within this large taxon, the genus *Dracoderes* appeared as the sister clade to Homalorhagida or even nested amongst the homalorhagid genera, as an ingroup.

Therefore and according to modern phylogenetic analysis including molecular and morphological datasets, the updated classification of the phylum divides the kinorhynchs into two major clades, now at the class level: Cyclorhagida and Allomalorhagida. The latter is a new assemblage of taxa that accommodates the traditional homalorhagid genera (*Pycnophyes*, *Kinorhynchus*, *Paracentrophyes*, *Neocentrophyes*, *Mixtophyes*) together with *Dracoderes* and *Franciscideres*, the morphotype of the two latter resembling the classical cyclorhagids. The traditional taxon Homalorhagida has been rejected as a taxonomic and phylogenetic unit due to its paraphyletic or polyphyletic nature.

Adittionally, this phylogenetic study stressed that the internal relationships of some taxa, mainly those of the two largest kinorhynch families, Echinoderidae and Pycnophyidae, are still unresolved. The family Pycnophyidae included traditionally two genera, *Pycnophyes* and *Kinorhynchus.* All recent phylogenetic studies recover the species of *Kinorhynchus* nested amongst the species of *Pycnophyes*, leaving as polyphyletic and paraphyletic groups respectively. Both genera share the same external morphology, just differing in the lateral terminal spines, absent in *Kinorhynchus* and present in *Pycnophyes*.

Despite the great effort in the kinorhynch research done during the recent years, the knowledge on these animals is far from complete. This fact is a result, at least somehow, of the very

small community of researchers interested on the phylum, currently less than ten people worldwide. Therefore the whole picture of the extant diversity is still unrevealed. The current knowledge of global kinorhynch biogeography seems likely to be strongly influenced by the sampling bias, fitting better with a sampling distribution chosen by the scientists than to the real distribution of the animals. Even within the relatively well-investigated areas, such as the North American East Coast, the Korean Peninsula, the European West Coast and the Mediterranean Sea, the discovery of new species or new reports is still frequent.

Main objectives

Given the current outlook on kinorhynchs and on the allomalorhagids in particular, several aspects or fields need further and deeper investigations. Therefore, the main goal of the present Thesis is to contribute to the huge task of increasing our general knowledge of the biology of Kinorhyncha, through the scopes of taxonomy, biogeography, morphology and phylogeny, giving an integrating view of these approaches. The objectives of the present work may be summarized as follows:

- To standardize an accurate terminology for the specific morphological characters of Allomalorhagida and their position along the trunk.
- To Identify the Allomalorhagida species collected in the sampled areas to the lowest taxonomic level.
- To describe new Allomalorhagida taxa from the sampled areas.
- To provide a detailed geographic distribution of Allomalorhagida from the Iberian Peninsula.
- To suggest new phylogenetic hypotheses for the Allomalorhagida.
- To test the monophyly of the genera *Pycnophyes* and *Kinorhynchus*.
- To shed light into the internal relationships of Pycnophyidae for the first time.
- To stablish the evolutionary pathway of morphological characters with phylogenetic relevance.

Main results of the research

Taxonomy

The first result of the present work is to stablish a new and accurate terminology for the specific morphological characters of Allomalorhagida and their position along the trunk. This purpose required the observation of a huge amount of Allomalorhagida specimens with the aim of selecting the most objective characters and standardizes their positions along the trunk, fitting with the typical triangular transverse section of allomalorhagids. Moreover, many specific morphological characters

were reevaluated and redefined for the different families. This new terminology and definitions will help taxonomic studies enabling us to perform precise and homogeneous, comparable descriptions.

Eleven new species of Allomalorhagida have been described following the established standards of the group. Ten of these species were accommodated into Pycnophyidae whereas only one, *Mixtophyes abyssalis*, was accommodated into Neocentrophyidae, resulting on the first description of an allomalorhagid genus since more than 30 years ago. The description of these eleven species, together with *Pycnophyes norenburgi* (also described by our team, as *P.* sp11, but under revision), increased the valid species of Pycnophyidae worldwide by a 20%, bringing the total number from 45 up to 56 species.

Morphology

The description of some of the new taxa together with a deep knowledge on the morphology of group, allowed us to pay attention to unusual cuticular features sometimes not noticed previously, such as the keel-shaped middorsal processes, the absence of male-specific tubes in Pycnophyidae or the organization of segments 1 and 11 in Neocentrophyidae. The observation of these uncommon characters gave rise to new hypotheses on their relationships and on the body plan evolution. Some of these hypotheses were later confirmed by phylogenetic studies under total evidence analyses, such is the plesiomorphic condition of lateral terminal spines and the arrangement of segment 1 as a complete ring in Allomalorhagida; and the phylogenetic relevance of the middorsal specializations whose character state evolution was traced in the combined tree. Contrarily, some other hypotheses were rejected through the total evidence analyses, as the potential monophyly of a group of *Pycnophyes* species without male-specific tubes.

A new technique, the Micro Computed Tomography (Micro-CT), has been applied for the first time to the study of kinorhynchs. Being the results preliminary, the technique has revealed a completely new field or morphological research, with a great potential for integrative studies on functional morphology. The fine tuning of Micro-CT techniques to be applied to so tiny animals can also be of advantage for other meiofaunal groups.

Phylogeny

According to our total evidence analyses neither *Pycnophyes* nor *Kinorhynchus* were recovered as monophyletic groups. Therefore, *Kinorhynchus* was rejected as a valid taxonomic and phylogenetic unit and no longer should be considered an allomalorhagid genus. Accordingly, *Pycnophyes* has been redefined with new diagnostic characters. The lateral terminal spines (or their

rudimental stage), which basically is the single difference between both genera, resulted to be homoplastic and without phylogenetic relevance.

Subsequently, a new classification of Pycnophyidae based on natural groups was established. The phylogenetic analyses yielded nine clades supported by morphological and molecular apomorphies and hence all of them have been erected as new genera.

Biogeography

Four species were described from the Iberian Peninsula (the first report of Pycnophyidae from the area), four from the Korean Peninsula, and three from the Guinea Basin deep-sea. Beside the new species for science, several already known species and additional undescribed species were reported: five from Italy, three from USA, seven from Panama, five from Norway, three from Greenland and one for Singapore.

The high diversity found along the Iberian Peninsula deserves a special mention: a total of 15 Allomalorhagida species were reported from 122 sampling localities. Notably, the species composition shows that most southern Mediterranean localities have a kinorhynch fauna more similar to the Atlantic rather than the Mediterranean, being strongly influenced by the Atlantic waters.. This pattern on the kinorhynch diversity may be a result of the Atlantic surface current.

Furthermore, both the newly described species and the findings reported herein from different regions contribute to improve the current knowledge on the worldwide geographic distribution of kinorhynchs, even though there is still an immense gap to fill until completing the goal.

RESUMEN

Conceptos básicos

El filo Kinorhyncha está constituido por invertebrados meiobentónicos exclusivamente marinos y de vida libre. En la actualidad, el filo comprende aproximadamente de 200 especies y forma parte del gran conjunto de los Ecdysozoa. Los kinorrincos, también llamados comúnmente dragones del fango, son de pequeño tamaño, entre 0.13 y 1.04 mm, lo que les permite habitar los espacios intersticiales que quedan entre las partículas de sedimento marino, ya sea fango o arena gruesa. Se encuentran fundamentalmente en los primeros centímetros del sedimento, donde el contenido de oxígeno es mayor, desde zonas costeras e intermareales hasta regiones oceánicas profundas. Ocasionalmente se han encontrado individuos sobre algas, sustratos duros o asociados como fauna epibionte a gran variedad de invertebrados sésiles.

Actualmente, el filo Kinorhyncha se incluye dentro del grupo monofilético Scalidophora, junto con los filos Priapulida y Loricifera. Scalidophora y Nematoida (formado por Nematoda más Nematomorpha) constituyen los Cycloneuralia. Dicho grupo está bien definido por apomorfías morfológicas, como la existencia un cerebro en forma de collar que rodea a la faringe; sin embargo, su monofilia ha sido rara vez apoyada por estudios moleculares. A su vez, los Cycloneuralia junto con los Panartophoda (Tardigrada, Onychophora y Arthropoda) forman el grupo Ecdysozoa, cuya monofilia está bien fundamentada tanto por caracteres moleculares como morfológicos, como por ejemplo la presencia de una cutícula no ciliada que se muda periódicamente y cuyo control está regulado supuestamente por hormonas ecdisteroides.

El patrón morfológico de los kinorrincos es muy homogéneo en términos generales. El cuerpo, alargado y cubierto por cutícula, queda dividido en tres regiones: cabeza, cuello y tronco. La cabeza está formada por un introverto eversible y un cono bucal protrusible. El cuello consiste en un anillo de placas rígidas, llamadas plácidas, las cuales actúan como sistema de cierre cuando el introverto y el cono bucal quedan completamente retraídos dentro del cuerpo del animal. El tronco está formado por once segmentos, que pueden estar divididos en placas cuticulares o bien formar anillos cerrados. Además, cada segmento puede presentar diversas estructuras cuticulares externas cuyo tipo, número y disposición se han utilizado tradicionalmente como caracteres taxonómicos. La cabeza y el cuello no se consideran segmentos pero la segmentación de los kinorrincos se hace evidente en el tronco, tanto en la disposición de los órganos internos (sistema nervioso, sistema muscular y glándulas epidérmicas) como en las estructuras cuticulares externas (placas, espinas, sedas, tubos y órganos sensoriales).

La clasificación del grupo se estableció de acuerdo con su morfología externa y la organización de las placas en cada segmento, especialmente los dos primeros, en los que la disposición está directamente relacionada con el sistema de cierre del tronco. Sin embargo, y a pesar de que las clasificaciones tradicionales están basadas en un conocimiento profundo de la morfología de los kinorrincos, no reflejan la evolución interna del grupo sino que se basan únicamente en aspectos fenéticos y no siguen los criterios establecidos por Hennig. Por lo tanto las relaciones filogenéticas dentro del filo que se han ido sugiriendo a lo largo de los años han estado erróneamente influidas por la clasificación taxonómica, cuando en realidad el proceso debería ser inverso, es decir, que la clasificación de un grupo esté inferida a partir de un árbol filogenético.

Según la composición de los segmentos y la anotomía externa general, los kinorrincos se han organizado tradicionalmente en dos grandes órdenes: Homalorhagida y Cyclorhagida. El orden Homalorhagida comprende un único suborden (Homalorhagae), mientras que el orden Cyclorhagida se divide en tres según el sistema de cierre: Conchorhagae (cierre bilateral, en forma de concha), Criptorhagae (sistema de cierre radial y no mediado por plácidas) y Cyclorhagae (sistema de cierre radial y mediado por plácidas).

La descripción de nuevos taxones se ha ido incorporando de una manera más o menos forzada a esta clasificación general, ampliamente aceptada hasta la aparición de los primeros trabajos de filogenia molecular centrados en el filo, con conclusiones sorprendentes. Los resultados moleculares han sido en gran medida congruentes con los grupos de la clasificación tradicional y esclarecieron muchas de las relaciones entre los grandes clados dentro del filo. Sin embargo, estos estudios también revelaron la existencia de algunos clados nunca propuestos anteriormente. La principal novedad de los resultados moleculares frente a la clasificación tradicional fue la polifilia de los Cyclorhagida. Dentro de este gran taxón, el género *Dracoderes* apareció como grupo hermano del orden Homalorhagida o incluso anidado entre los géneros de homalorrágidos, como un grupo interno.

Por lo tanto y basándonos en los resultados de los análisis filogenéticos que incluyen datos morfológicos y moleculares, la clasificación actualizada del filo divide a los kinorrincos en dos grandes clases: Cyclorhagida y Allomalorhagida. Este último es un nuevo ensamblaje de taxones que incluye a los tradicionales géneros de homalorrágidos (*Pycnophyes*, *Kinorhynchus*, *Paracentrophyes*, *Neocentrophyes* y *Mixtophyes*) junto con *Dracoderes* y *Franciscideres*, cuyos morfotipos recuerdan más al de los clásicos ciclorrágidos. Además, el tradicional taxón Homalorhagida ha sido rechazado como unidad taxonómica y filogenética debido a su naturaleza parafilética o polifilética según los análisis.

A pesar de los grandes esfuerzos llevados a cabo en los últimos años en la investigación de los kinorrincos, el conocimiento de estos animales es aún muy escaso. Este hecho se debe, al menos en parte, a la reducida comunidad de investigadores interesados en el filo, actualmente con menos de 10 profesionales dedicados a la materia en todo el mundo. Por lo tanto, el panorama general sobre la diversidad del grupo está aún por descubrir en su mayor parte. El conocimiento actual en cuanto a la distribución mundial del grupo parece estar altamente influido por el sesgo de muestreo, de manera que refleja más una distribución de los puntos de muestreo que la distribución real de los animales. El descubrimiento de nuevas especies o nuevas citas es frecuente incluso dentro de las áreas consideradas como relativamente bien estudiadas, como la costa este de Norteamérica, la Península Coreana, la costa oeste de Europa y el mar Mediterráneo.

Objetivos

Dado el estado actual de los estudios sobre los kinorrincos en general y de los alomalorrágidos en concreto, existen varios aspectos de su biología que precisan ser abordados con mayor detalle. Así, el principal objetivo de la presente Tesis Doctoral es contribuir a la enorme tarea de aumentar el conocimiento de los kinorrincos en cuanto a taxonomía, biogeografía, morfología y filogenia se refiere, aportando una visión integradora de estas disciplinas. Los objetivos de este trabajo se pueden resumir de la siguiente manera:

- Establecer y estandarizar una terminología adecuada de los caracteres morfológicos de los Allomalorhagida así como de las posiciones de los mismos a lo largo del tronco.
- Identificar las especies de Allomalorhagida obtenidas en las áreas de muestreo.
- Describir los nuevos taxones de Allomalorhagida encontrados las áreas muestreadas.
- Aportar una distribución geográfica lo más detallada posible de los Allomalorhagida en la Península Ibérica.
- Sugerir nuevas hipótesis filogenéticas para los Allomalorhagida.
- Testar la monofilia de los géneros *Pycnophyes* y *Kinorhynchus*.
- Arrojar luz por primera vez sobre las relaciones internas de la familia Pycnophyidae.
- Trazar la serie de transformación evolutiva de los caracteres morfológicos con relevancia filogenética dentro del grupo.

Resultados principales de la investigación

Taxonomía

El primer resultado del presente trabajo de investigación fue el asentamiento de la terminología de los caracteres morfológicos de los alomalorrágidos y sus posiciones en el organismo, de la manera más adecuada y precisa posible. Dicho propósito precisó de la observación de una enorme cantidad de ejemplares de Allomalorhagida con el fin de seleccionar los caracteres más objetivos y estandarizar sus posiciones a lo largo del tronco de acuerdo con la sección transversal típicamente triangular de los alomalorrágidos. Además, muchos caracteres morfológicos específicos de las diferentes familias fueron reevaluados y redefinidos. Esta nueva terminología contribuirá a la elaboración de futuros estudios taxonómicos ya que permite desarrollar descripciones precisas, homogéneas y comparables.

Se han descrito once nuevas especies de Allomalorhagida de acuerdo con los estándares establecidos para el grupo. Diez de estas especies fueron asignadas a la familia Pycnophyidae mientras que sólo una de ellas, *Mixtophyes abyssalis*, fue asignada a la familia Neocentrophyidae. El descubrimiento de dicha especie ha supuesto la primera descripción de un género de Allomalorhagida desde hace más de 30 años. La descripción de las once especies, junto con *Pycnophyes norenburgi* (descrito por nuestro equipo, y también *P. sp11*, en estado de revisión), incrementó las especies válidas de Pycnophyidae a nivel mundial en un 20%, elevando el número total de especies de 45 a 56.

Morfología

La descripción de algunos de los nuevos taxones junto con un conocimiento profundo de la morfología del grupo reveló la existencia de determinados caracteres cuticulares poco comunes, cuya presencia no había sido valorada anteriormente de forma adecuada, como por ejemplo los salientes mediodorsales en forma de quillas, la ausencia de los tubos específicos de los machos de Pycnophyidae o la organización de los segmentos 1 y 11 en Neocentrophyidae. La observación de estos caracteres poco comunes dio lugar a nuevas hipótesis sobre las relaciones dentro del grupo y sobre la evolución de los planes corporales. Algunas de estas hipótesis fueron confirmadas posteriormente mediante estudios filogenéticos de evidencia total, como pueden ser la presencia de espinas lateroterminales y la organización del segmento 1 en forma de anillo completo como condiciones plesiomórficas en la clase Allomalorhagida. Así mismo, se confirmó la importancia filogenética de las especializaciones mediodorsales, cuya evolución de los estados del carácter se rastreó en el árbol filogenético combinado. Por el contrario, otras hipótesis fueron rechazadas tras el

análisis de evidencia total, como la monofilia del supuesto grupo de especies de *Pycnophyes* sin tubos ventrales en el segmento 2 de los machos.

Se ha aplicado por primera vez en el estudio de los kinorrincos la nueva técnica de la Micro Tomografía Computarizada (Micro-CT). Aunque los resultados son preliminares, ésta técnica se ha revelado como un nuevo campo para la investigación morfológica, de gran potencial para estudios integradores de la morfología funcional. La estandarización de las técnicas del Micro-CT en animales de tan reducido tamaño supone una gran ventaja para poder trabajar en el futuro con otros grupos de la meiofauna.

Filogenia

Según nuestros resultados de evidencia total, ni *Pycnophyes* ni *Kinorhynchus* resultaron ser grupos monofiléticos. Por lo tanto, *Kinorhynchus* fue rechazado como unidad taxonómica y filogenética y no debe ser considerado nunca más como un género de alomalorrágidos. La ausencia de espinas laterotерminales (o la presencia de un estado rudimentario de las mismas), que básicamente es la única diferencia entre ambos géneros, resultó ser homoplásica y carente de relevancia filogenética.

Subsecuentemente, se estableció una nueva clasificación de la familia Pycnophyidae basada en grupos naturales. Los análisis filogenéticos produjeron nueve clados, cada uno apoyado por apomorfías moleculares y morfológicas que en consecuencia han sido erigidos como nuevos géneros.

Biogeografía

Se describieron cuatro especies de la Península Ibérica (primera mención sobre la presencia de la familia Pycnophyidae en la zona), cuatro de la Península Coreana y tres de la cuenca profunda de Guinea. Aparte de las once nuevas especies para la ciencia incluidas en esta Tesis, se encontraron especies ya conocidas y otras aún sin describir procedentes de Italia, EEUU, Panamá, Noruega, Groenlandia y Singapur.

La gran diversidad encontrada a lo largo de las Península Ibérica merece una mención especial: se encontraron un total de 15 especies de Allomalorhagida procedentes de 122 localidades de muestreo. Cabe destacar que la composición de especies de las localidades mediterráneas localizadas más al sur es más similar a la composición de las localidades atlánticas que a la de las mediterráneas. Así, las localidades mediterráneas del sur parecen estar influidas por la fuerte corriente superficial de aguas atlánticas.

Asimismo, tanto las nuevas especies descritas recientemente como los hallazgos de especies en las distintas localidades recopilados aquí contribuyen a la mejora del conocimiento de la distribución geográfica de los kinorrincos a nivel mundial, aunque el objetivo final está aún lejos de ser alcanzado.

LIST OF COMPILED PUBLICATIONS

The present Doctoral Thesis is composed of six publications and two Appendixes with unpublished and complementary material, all of them included in the *Results* section.

Chapter I: Taxonomy of Pycnophyidae

- Nuria Sánchez, Fernando Pardos, María Herranz, Jesús Benito. ***Pycnophyes dolichurus* sp. nov. and *P. aulacodes* sp. nov. (Kinorhyncha, Homalorhagida, Pycnophyidae), two new kinorhynchs from Spain with a reevaluation of homalorhagid taxonomic characters.** *Helgoland Marine Research*, 65: 319-334.
- Nuria Sánchez, María Herranz, Jesús Benito, Fernando Pardos. ***Pycnophyes almansae* sp. nov. and *Pycnophyes lageria* sp. nov., two new homalorhagid kinorhynchs (Kinorhyncha, Homalorhagida) from the Iberian Peninsula, with special focus on introvert features.** *Marine Biology Research*, 10: 17-36.
- Nuria Sánchez, Hyun Soo Rho, Won-Gi Min, Dongsung Kim, Martin Vinther Sørensen (2013). **Four new species of *Pycnophyes* (Kinorhyncha: Homalorhagida) from Korea and the East China Sea.** *Scientia Marina*, 77: 353-380.
- Nuria Sánchez, Fernando Pardos, Martin V. Sørensen (2014). **Deep-sea Kinorhyncha: two new species from the Guinea Basin, with evaluation of an unusual male feature.** *Organism Diversity and Evolution*, 14: 349-361.

Chapter II: Taxonomy of Neocentrophyidae

- Nuria Sánchez, Fernando Pardos, Martin V. Sørensen (2014). **A new kinorhynch genus, *Mixtophyes* (Kinorhyncha: Homalorhagida), from the Guinea Basin deep-sea, with new data on the family Neocentrophyidae.** *Helgonald Marine Research*, 68: 221-239.

Chapter III: Phylogeny

- Nuria Sánchez, Hiroshi Yamasaki, Fernando Pardos, Martin V. Sørensen, Alejandro Martínez (accepted). **Morphology disentangles the systematics of a ubiquitous but elusive meiofaunal group (Kinorhyncha: Pycnophyidae).** *Cladistics*.

Appendix I: Morphology

- Kinorhynchs through Micro-CT (Kinorhyncha: Allomalorhagida): a new window into the meio-world.
- Morphological matrix of Pycnophyidae and Neocentrophyidae.
- Morphological character description of Pycnophyidae and Neocentrophyidae.

Appendix II: Geographical distribution

- New data on geographical distribution with special focus on the Iberian Peninsula.

ARRANGEMENT OF FIGURES AND TABLES

Figures and Tables in the *Introduction*, *Material and Methods* and *Appendixes* are numbered independently from the Figures and Tables inside the publications (included in the *Results* section), which follow the arrangement and style of their respective journals.

Chapter III: Table 4 of the publication contained within this chapter is included in the section *Appendix I: Morphology*, tittled as *Morphological matrix of Pycnophyidae and Neocentrophyidae*. Each of the morphological characters referred to in the matrix are accurately definided within the same Appendix and following the same numbering.

1

INTRODUCTION

The oceans cover most of the surface of our planet and represent approximately 95% of the biosphere. Sea water is the living medium of a great animal diversity; with representatives of 32 out of the 34 extant animal phyla, of which 13 are exclusively or mostly marine. Most of the known fauna of the oceans belongs to the relatively well-studied plankton and nekton realms, in contrast to the benthos diversity, still mainly unexplored. The studies on the benthos diversity have been mostly focused in the macrobenthos, whereas the organisms of small size, the meiobenthos or meiofauna, are still poorly known. The meiofauna is composed by the pool of organisms that live between sediment particles in marine bottoms, with a body length from 0.45 to 1 mm. Most of the currently recognized animal phyla have meiofaunal representatives, but only five are exclusively meiofaunal (Higgins and Thiel, 1988; Giere, 2009).

Kinorhyncha (Greek “kineo”, move; “rhynchos”, snout) is one of these exclusively meiobenthic phyla. Kinorhynchs, or mud dragons, are free-living marine ecdysozoan invertebrates, comprising around two hundred described species at present with body lengths ranging from 0.13 to 1.04 mm. Kinorhynchs inhabit the interstices between grains of muddy to coarse sand sediments, mostly in the first, well oxygenated centimeters, from the intertidal zone to the deep sea bottoms. Occasionally, kinorhynchs have been found on algae or associated to a variety of invertebrates, such as bryozoans, hydroids, ectoprocts or poriferans (Higgins and Thiel, 1988; Sørensen and Pardos, 2008; Neuhaus, 2013; Sørensen 2013). Currently, the phylum is included within the Ecdysozoan ensemble, forming the Scalidophora together with Priapulida and Loricifera (Dunn et al., 2014).

All kinorhynchs share a similar external morphology, with an elongated body covered by cuticle and divided into three major regions: head, neck and trunk (Higgins and Thiel, 1988; Sørensen and Pardos, 2008; Neuhaus, 2013; Sørensen 2013). The head is reduced and bears an eversible introvert and a protrusible mouth cone, both with cuticular appendages radially arranged. The neck consists of a ring of plates named placids, which form a closing system when the introvert is completely retracted. The bilateral trunk is elongated and formed by eleven segments, externally visible by the arrangement of the cuticle, which may either be divided into cuticular plates or form a

closed ring (Higgins, 1990; Adrianov and Malakhov, 1999a; Sørensen and Pardos, 2008; Neuhaus, 2013). The segmentation is reflected in the anatomy of the trunk, both externally (plates, spines, setae, tubes, sensory spots) and internally (nervous and muscular systems and epidermal glands), whereas the head and neck are not considered as segmentary units (Brusca and Brusca, 2003; Sørensen and Pardos, 2008; Neuhaus, 2013). According to its external morphology and mainly based on the segment arrangement, Kinorhynchs have been traditionally accommodated into two large groups: Homalorhagida (Zelinka, 1896; Chitwood, 1951) (Allomalorhagida after Sørensen et al., in press) and Cyclorhagida (Zelinka, 1896) (Higgins, 1964, 1990; Adrianov and Malakhov, 1999a; Sørensen and Pardos, 2008; Neuhaus, 2013; Sørensen, 2013).

1.1. HISTORY OF RESEARCH ON KINORHYNCHA

On the first of July, 1841, the French naturalist Felix Dujardin found a small animal in a preserved sample from Saint-Malo. Between 1841 and 1849 he found specimens displaying a similar morphotype working on oysters and he named it as *Échinodère* ("spine-neck") in a contribution titled "*Sur un petit animal marin*, *l'Échinodère*, *formant un type intermédiaire entre les Crustacés et les Vers*" (Dujardin, 1851). He noted resemblances of the new animal with nematodes, rotifers, sipunculans, acanthocephalans, tardigrades and several other invertebrate groups, and considered its place in the animal system somewhere between crustaceans and worms. In 1863, the French zoologist E. Claparède reported several species, including *Echinoderes dujardinii* in honor of its discoverer (Claparède, 1863).

The first extensive works were made by Greeff and Metschnikoff, who described species from the Canary Islands (Spain) and Ostende (Belgium) and Salerno (Italy), respectively (Greeff, 1869; Metschnikoff, 1869). In 1875, Pagenstecher described a new species from the Balearic Islands (Spain), which was later synonimized with *Echinoderes dujardinii* (Pagenstecher, 1875). The next year, Panceri (1876) also described several species from Italy but most of them were also synonimized with *E. dujardini* in the end. Reinhard (1881, 1885, 1887) was the first researcher that reported kinorhynchs from the Black Sea, describing nine species, including *Echinoderes dentatus* which later became *Pycnophyes dentatus*. In addition, Reinhard was the first author that referred to the group as "Kinorhyncha" in his monograph (Reinhard, 1885, 1887), justifying the use of the new term and thus, Kinorhyncha was established as an alternative name to Echinodera.

After the initial researches, several authors expressed their interest in the kinorhynchs, such as Zelinka (1894), but their contributions were limited to just a mere mention of the presence of the group or to report *Echinoderes dujardinii* in additional localities. It was not until 1896 when Zelinka

established a classification mainly based on the closing system of the trunk, leaving the species accommodated into two orders, Homalorhagae and Cyclorhagae. Homalorhagae with three new families: Pycnophyidae, Centrophyidae, and Trachydemidae; and Cyclorhagae contained the family Echinoderidae and a new family, Centroderidae Zelinka (1896). Several years later, Zelinka (1907) established additional new taxa: the new order Conchorhagae accomodating the new family Pentacontidae and the genus *Semnoderes*; two new suborders, Nomostomata and Xenostomata, belonging to the Cyclorhagae, each one with one new family, Centroderidae and Mesitoderidae, respectively; and the new genera *Trachydemus* (Trachydemidae), *Pycnophyes* (Pycnophyidae), *Echinoderella* (Echinoderidae), *Campyloderes* (Mesitoderidae) and *Centroderes* (Centroderidae). The same year, Schepotieff published the most extensive review on kinorhynchs to date (Schepotieff, 1907). Subsequently, Southern (1914) carried out an extensive faunistic survey along the Northwest coast of Ireland, Clew Bay. He described three new species of kinorhynchs and reported several already described, such as *Pycnophyes dentatus* and *Echinoderes dujardinii*.

Few years later, Zelinka published his "*Monographie der Echinodera*" (Zelinka, 1928). This outstanding contribution is still a reference work for the current kinorhynch researches. In his monograph, he described 16 species still valid today, from the Mediterranean Sea and the Atlantic European coast. This research provided data on the internal anatomy and biology of the group and stablished at the same time many of the current terminology, bringing up the kinorhynchs to the research world.

Studies on kinorhynchs were scarce during the next decades. Several authors contributed to the kinorhynch knowledge by isolated description of species (Abe, 1930; Blake, 1930; Remane, 1936; Lou, 1934; Johnston, 1938; Chitwood, 1951; Hyman, 1951; Karling, 1955; Omer-Cooper, 1957), or genera (Gerlach, 1956) or were focused on the embryological development of the group (Nyholm 1947a, 1947b, 1947c). It was not until the sixth decade and forth when the kinorhynch taxonomy reached a remarkable breakthrough thanks to R.P. Higgins and coworkers, who described more than 60 species and six genera (Higgins, 1960, 1961, 1964a, 1964b, 1965, 1966a, 1966b, 1967, 1968, 1969a, 1969b, 1977a, 1977b, 1978, 1983, 1985, 1986a, 1986b, 1990; Higgins and Adrianov, 1991; Higgins and Korczynski, 1989; Higgins and Kristensen, 1988; Higgins and Rao, 1979; Higgins and Shirayama, 1990; Martorelli and Higgins, 2004; Pardos et al., 1998). Moreover, R.P. Higgins improved the techniques for the kinorhynch extraction from the sediment, standardized the taxonomical descriptions and redescribed many species with modern criteria (Higgins and Thiel, 1988; Higgins, 1983). Furthermore, he stablished most of the terminology used in kinorhynch taxonomy and the precise location of taxonomical characters along the body.

Since the end of the XX century, most of the contributions to the taxonomy of the group were carried out through extensive studies made by M.V. Sørensen, A. Adrianov, B. Neuhaus, F. Pardos and coworkers (Adrianov, 1989, 1995; GªOrdóñez et al., 2008; Pardos et al., 1998; Adrianov and Malakhov, 1999a; Song and Chang, 2001; Sørensen et al., 2005, 2007, 2010a, 2010b, 2010c, 2010d, 2012a, 2012b, 2013; Neuhaus, 2004; Neuhaus and Blasche, 2006; Sørensen, 2006, 2008; Sørensen and Rho, 2009; Sørensen and Thormar, 2010; Herranz et al., 2012; Neuhaus and Sørensen , 2012; Neuhaus et al., 2013; Yamasaki and Kajihara, 2012; Yamasaki et al., 2012; Dal Zotto et al., 2013). Nevertheless, this vast contribution was mainly focused on the Cyclorhagida, both for the descriptions of species and genera. Most of the new genera were undoubtedly accommodated into the Cyclorhagida but some of them were not assigned to any family due to their special features and left as *incertae sedis* (Sørensen et al., 2007; Sørensen and Thormar, 2010; Dal Zotto et al., 3013). These odd genera opened new questions and hypotheses about the internal relationships into the phylum and hence the whole systematic arrangement has been questioned.

In addition to the studies on the diversity and taxonomy of the phylum, several studies were carried out to improve the knowledge on other aspects of kinorhynch biology such as the internal anatomy. The gross body plan and organ system arrangement was already advanced by Reinhard (1885, 1887) and Zelinka (1928) through LM observations. It was not until the last two decades of the XX century when the Electron Microscopy, both Transmission (TEM) and Scanning (SEM) were applied to the study of kinorhynchs (Higgins, 1983; Neuhaus, 1988, 1991, 1994, 1997; Adrianov et al., 1989, 1990; Adrianov and Malakhov, 1990, 1991, 1994, 1999b; Kristensen and Higgins, 1991; Nebelsick, 1992a-b, 1993; Pardos et al., 1998). With the new century, the Confocal Laser Scanning Microscopy (CLSM) open new research fields for the study of kinorhynch anatomy, mainly focused on the muscular and nervous systems (Müller and Schmidt-Rhaesa, 2003; Rothe and Schmidt-Rhaesa, 2004; Schmidt-Rhaesa and Rothe, 2006; Herranz et al., 2013, 2014a).

1.2. KINORHYNCHA MORPHOLOGY

1.2.1. External morphology

The **head** comprises the protrusible mouth cone, bearing the mouth, and the eversible introvert (Fig. 1). The mouth cone has cuticular appendages named oral styles. Four rings of oral styles can be distinguished: the three most internal rings are formed by the inner oral styles (5, 5 and 10 styles, from inner to outer) and the most external one is formed by nine outer oral styles, being the middorsal one missing (Figs. 1-2). The outer oral styles may show a high variability among taxa: they may be all of similar sizes or alternate between larger and smaller; consist of a single long and flexible piece, or are composed of robust, rigid, articulated pieces (Fig. 1). Moreover, some taxa show an alternate mixture of both kinds of oral styles, rigid and articulated and thin and flexible (Sørensen and Pardos, 2008; Neuhaus, 2013).

The *introvert* is formed by large appendages, arranged pentaradially in up to seven concentric circles around the mouth cone, which bears the oral styles (Figs. 1-2). Even though all the introvert appendages receive the common name “scalid”, we can discriminate three types: primary spinoscalids in ring 01, spinoscalids in rings 02–06, and trichoscalids in ring 07 (Neuhaus, 2013). Each spinoscalid consists of a short basal sheath, articulated with a long spinose distal end, which is blunt in ring 01 and pointed in rings 02–06(07). The ring 01 bears ten primary spinoscalids whereas the number of spinoscalids in rings 02-06 varies between five, ten, fifteen and twenty.Trichoscalids are always located in the last ring and these may be attached directly to the introvert or through sclerotized trichoscalid plates, depending on the families. Their number varies among 6, 9 or 14, and therefore they do not follow the pentaradial arrangement observed for spinoscalids. All trichoscalids have a wide and hairy base and a narrower terminal tip (Brown, 1989; Sørensen and Pardos, 2008).

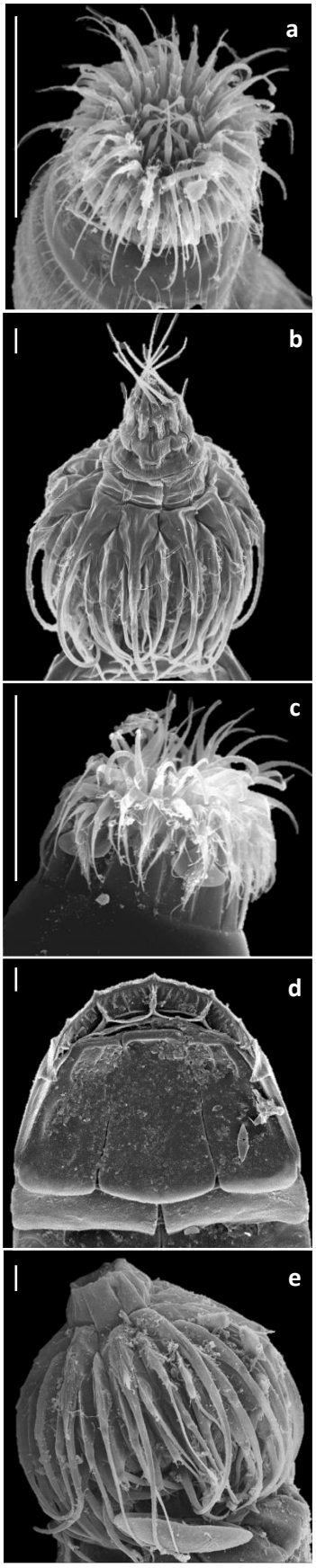
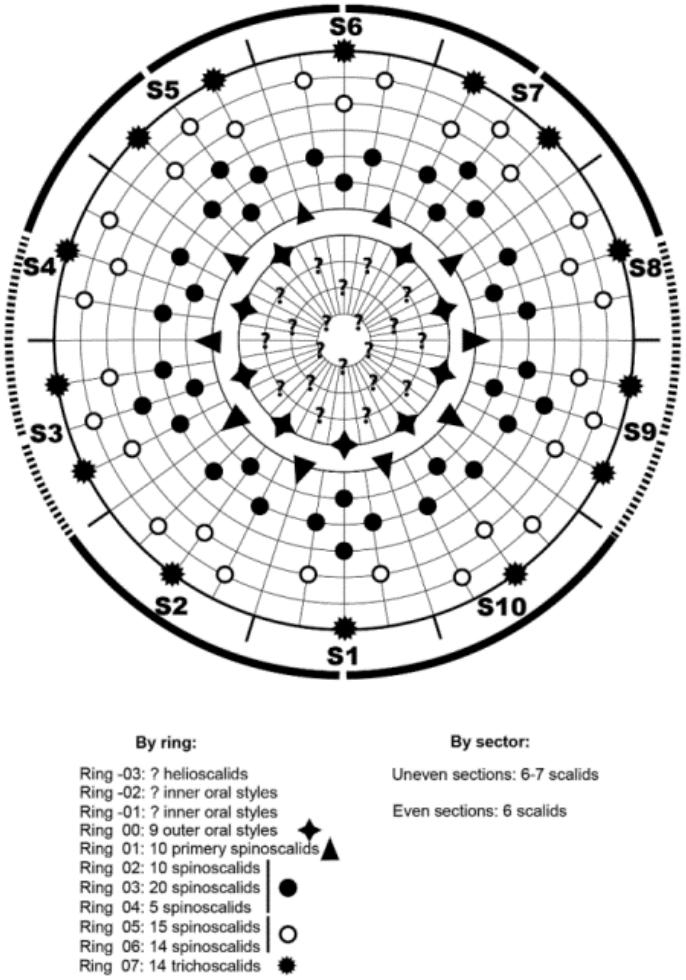


Figure 1. a, *Echinoderes cantabricus,* polar view of introvert showing articulated outer oral styles (scale bar: 60 µm); b, *Pycnophyes communis*, ventral view of the introvert and mouth cone with non-articulated and flexible outer oral styles (scale bar: 10 µm); c, *Echinoderes cantabricus*, placids and trichoscalid plates (scale bar: 40 µm); d, *Pycnophyes dentatus*, placids (scale bar: 10 µm); e, *Kinorhynchus* sp. introvert with an attached diatom (scale bar: 10 µm).

Other than by rings, the introvert can be divided by 10 radial sectors marked by the position of the primary spinoscalids and numbered clockwise from the midventral sector 1 (Fig. 2). Usually all even sectors show the same number and distribution of spinoscalids, different from the ones shared by the uneven sectors (Sørensen and Pardos, 2008; Neuhaus, 2013).



Ring/Sector		1	2	3	4	5	6	7	8	9	10	Total
00 oos	✦	1	1	1	1	1	0	1	1	1	1	9
01	▼	1	1	1	1	1	1	1	1	1	1	10
02	●	1	1	1	1	1	1	1	1	1	1	10
03	●	2	2	2	2	2	2	2	2	2	2	20
04	●	1	0	1	0	1	0	1	0	1	0	5
05	○	2	1	2	1	2	1	2	1	2	1	15
06	○	0	2	1	2	1	2	1	2	1	2	14
07 tr	✹	1	1	2	1	2	1	2	1	2	1	14
Total scalids		7	7	9	7	9	7	9	7	9	7	88

Figure 2. Polar diagram of mouth cone, introvert and placids in *Pycnophyes aulacodes* with the distribution and type of scalids by ring and sector. Question marks denote the assumed positions of the inner oral styles, whose presence could not be confirmed. Dashed lines mark flexible cuticular lateral areas between placids. 'Double diamonds' are marked in the table with double lines. oos, outer oral styles; Tr, trichoscalid.

When de introvert retracts inside the trunk, the anterior end of the body should be closed somehow, a function that the rigid cuticle of the first segments makes difficult. The closure is made by a series of sclerotized cuticular plates, the *placids*, which form the **neck** (Fig. 1). They are articulated basally with the anterior edge of the first segment of the trunk in most kinorhynchs, although in some taxa they may appear fused with the first segment or even may be lacking. Number, shape, aspect and development of placids vary according to the genus: 16, 14, 9, 8 or 7; small/large, narrow/broad, trapezoidal/triangular, smooth/ knobby (see Fig. 3) (Sørensen and Pardos, 2008; Neuhaus, 2013).

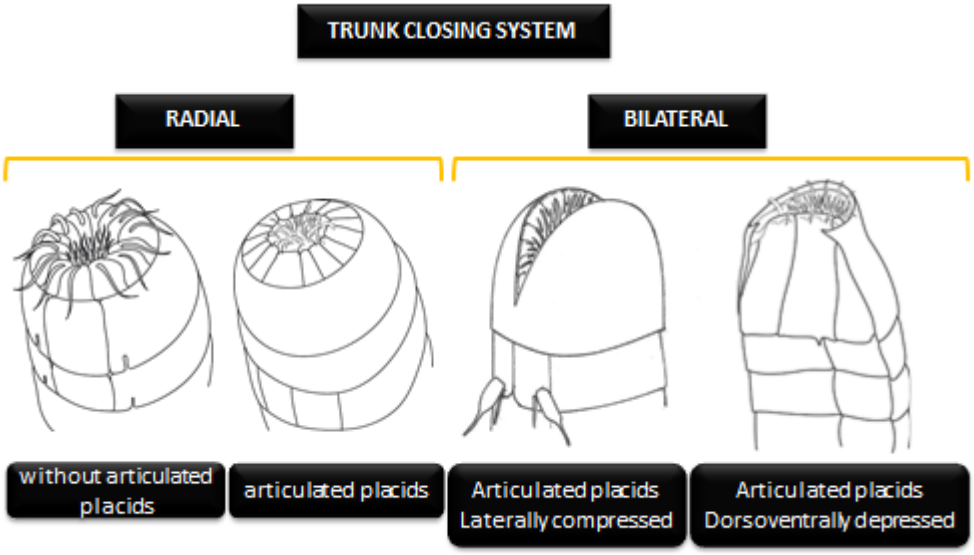


Figure 3. Different kinds of closing system in kinorhynchs, showing radial or bilateral symmetry of the closing system.

The elongated **trunk** consists of 11 segments in adults and the last juvenile stages, whereas the initial juvenile stages show a lower number of segments that increases through subsequent molts (Higgins, 1983; Neuhaus, 1993, 1995, 2013; Lemburg, 2002; Sørensen and Pardos, 2008; Sørensen et al., 2010b). The overall shape of the trunk (habitus) is commonly used as a taxonomical character since it differs between the two main groupings in the phylum (Sørensen and Pardos, 2008). The species of Allomalorhagida (Homalorhagida before Sørensen et al., in press) are generally bigger, with a nearly rectangular outline and a conspicuously triangular cross-section. By contrast, Cyclorhagida species have a slender, spindle-shaped body, with a rounded, heart-shaped or elliptical section (Fig. 4).

The cuticle of each segment may either be arranged as a closed ring or divide into dorsal (tergal) and ventral (sternal, usually two) cuticular plates (Sørensen and Pardos, 2008; Neuhaus, 2013). The arrangement of the cuticular plates by segment plays a key systematic role in the phylum, resulting

of a major taxonomical tool to discriminate amongst families and genera. In fact it is just the arrangement of cuticular plates in segments 1, 2 and 11 which changes among families and genera whereas the remaining segments are usually arranged into one tergal and two sternal plates (Sørensen and Pardos, 2008) (Fig. 4).

- *Composition of segment 1*: consisting of one closed ring in species of Cyclorhagida (hence their name), whereas most species of Allomalorhagida have the segment 1 divided into plates, with a variable arrangement in the ventral side depending on the families: a single sternal plate completely undivided, just partially divided anteriorly, or completely divided into three sternal plates (one midsternal and two episternal).
- *Composition of segment 2*: it may either consist on a closed ring or divide into plates in Cyclorhagida, whereas it is always divided into one tergal and two ventral sternal plates in most Allomalorhagida.
- *Composition of segment 11*: the sternal region of the terminal trunk segment divided into two plates is a widespread character amongst kinorhynchs, but it is composed of a single, undivided plate in few genera, both cyclorhagids and allomalorhagids.

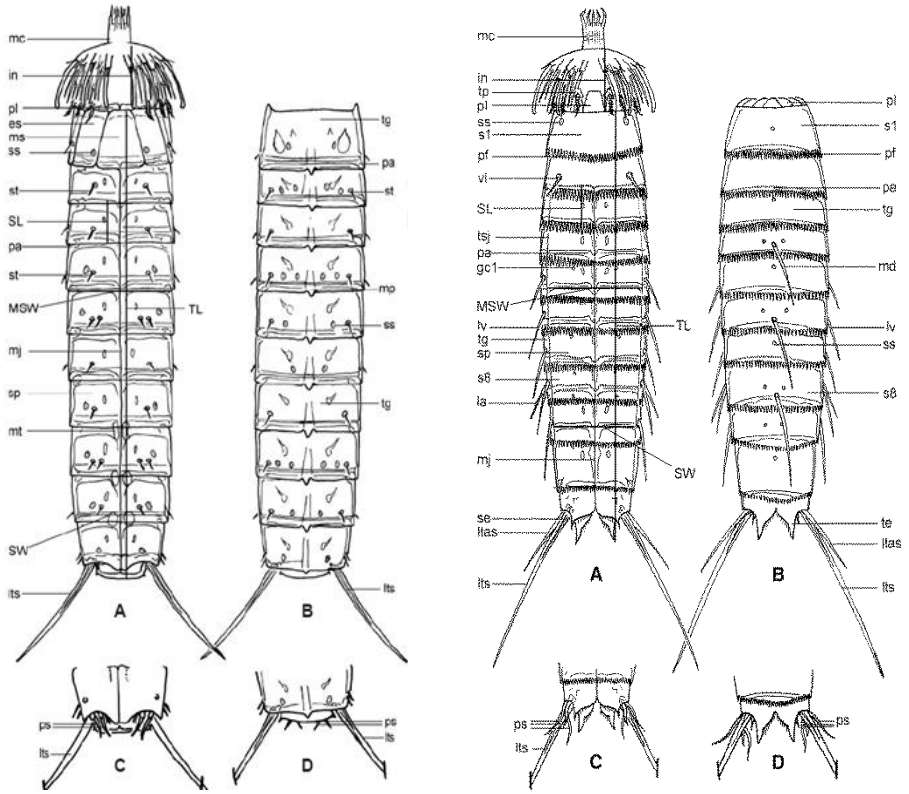


Figure 4. General external morphology of major kinorhynch groups: Allomalorhagida and Cyclorhagida; taxonomically relevant characters are shown. a, female, ventral view; b, female, dorsal view; c, detail of male posterior end, ventral view; d, detail of male posterior end, dorsal view. Abbreviations: es, episternal plate; in, introvert; lts, lateral terminal spine; mc, moth cone; mj, midsternal junction; mp, middorsal process; ms, midsternal plate; MSW, maximum sternal width; mt, apodeme; pa, pachycycli; pl, placid; ps, penile spine; SL, segment length; sp, sternal plate; ss, sensory spot; st, seta; SW, standard width; tg, tergal plate; TL, total trunk length. From Sørensen and Pardos, 2008.

Cuticular plates are articulated to each other: the tergal and sternal plates of the same segment are joined through lateral, *tergosternal junctions*, whereas the midventral junction joins the two sternal plates of each segment (Kristensen and Higgins, 1991; Sørensen and Pardos, 2008). Inner cuticular thickenings at the anterior edge of the segments, named *pachycycli*, are present in most kinorhynch families, serving as attachment sites for muscles; pachycycli often differentiate at the tergosternal junctions as specialized "ball and socket" joints (Fig. 4) (Kristensen and Higgins, 1991; Sørensen and Pardos, 2008).

Furthermore, each segment is equipped with several external cuticular structures whose type, number and arrangement are traditionally used as taxonomic characters for Kinorhyncha (Fig. 4). The distribution of such structures on the segment has prompted the development of a standardized system of positions to improve the accuracy and homogeneity of taxonomical descriptions (Pardos et al., 1998; Sánchez et al., 2011 in Chapter I), slightly differing between Cyclorhagida and Allomalorhagida (Fig. 5).

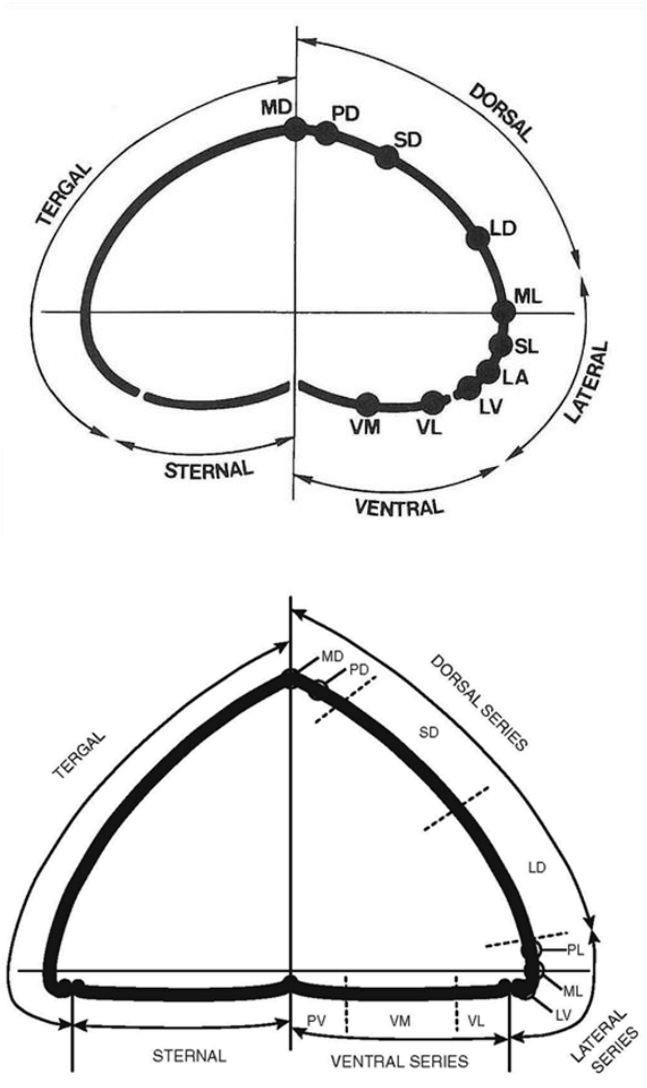


Figure 5. Drawing shows the most common cross sections through a trunk segment of a., Allomalorhagida; b, Cyclorhagida. Both drawings display positions of the cuticular characters. Lines are marked by circles. Bands are limited by dotted lines. Drawing of Cyclorhagida is taken from Pardos et al., 1998. Abbreviations: LA, lateral accessory; LD, laterodorsal; LV, lateroventral; MD, middorsal; ML, midlateral; PD, paradorsal; PL, paralateral; PV, paraventral; SD, subdorsal; SL, sublateral; VL, ventrolateral; VM, ventromedial.

The most conspicuous cuticular structures are described as follows:

1.2.1.1. Cuticular appendages: kinorhynchs bear several specialized cuticular equipment along the trunk (Fig. 6) (Sørensen and Pardos, 2008; Neuhaus, 2013; Sánchez et al. 2011 in Chapter I; Sánchez et al., 2014a in Chapter II) (Appendix 1).

- *Spines:* the term refers to cuticular appendages of the trunk formed by a single piece articulated proximally with the segment plate and a longer distal part ending in a pointed tip. Spines can be categorized into several types. *Acicular spines* are rigid or flexible, elongate spines with a closed tip; they typically occur at the middorsal line or the lateral region on some trunk segments (Fig. 7). Special, very robust and sometimes very long, acicular spines are present at the terminal segment: paired as the so-called *lateral terminal spines* or single as *midterminal spines* (Fig. 7). Middorsal and lateral acicular spines are present in all Cyclorhagida but are uncommon in Allomalorhagida (Figs. 6-7). The midterminal spine is an exclusive character of Cyclorhagida, diagnostic for the new taxon Kentrorhagata and the family Campyloderidae (Sørensen et al., in press). The presence of lateral terminal spines is a common character of kinorhynchs, absent in two Allomalorhagida genera only, which bear rounded, bulbous, articulated protuberances instead. *Cuspidate spines* are special cuticular appendages, pencil-shaped, attached to the segment plate by a broad basis that narrows distally to the end with a terminal opening (Fig. 7). This kind of spine occurs at the lateral region of a few cyclorhagid genera. The so-called *penile spines* are flexible appendages present bilaterally as two or three pairs between the two last segments in males of most kinorhynch genera. Their function is still uncertain.
- *Spinose processes:* the term refers to a non-articulated, pointed projection of the posterior edge of the tergal plate present in middorsal and lateral position. These have a conspicuous keel-shape, with an elongate base and a flexible terminal end, beginning at the anterior third of the segment and surpassing half of the following segment.
- *Middorsal processes:* protruding, usually hairy, structures that surpass well beyond the posterior margin of the segment along the middorsal trunk line. They may vary from just a pointed protrusion of the posterior segment margin to a clear keel shape with a rigid terminal end.
- *Middorsal elevations:* cuticular rounded or blunt structures poorly developed, not protruding beyond the segment margin. Usually seen as a bulging area, hump-like, flanked by sensory spots.

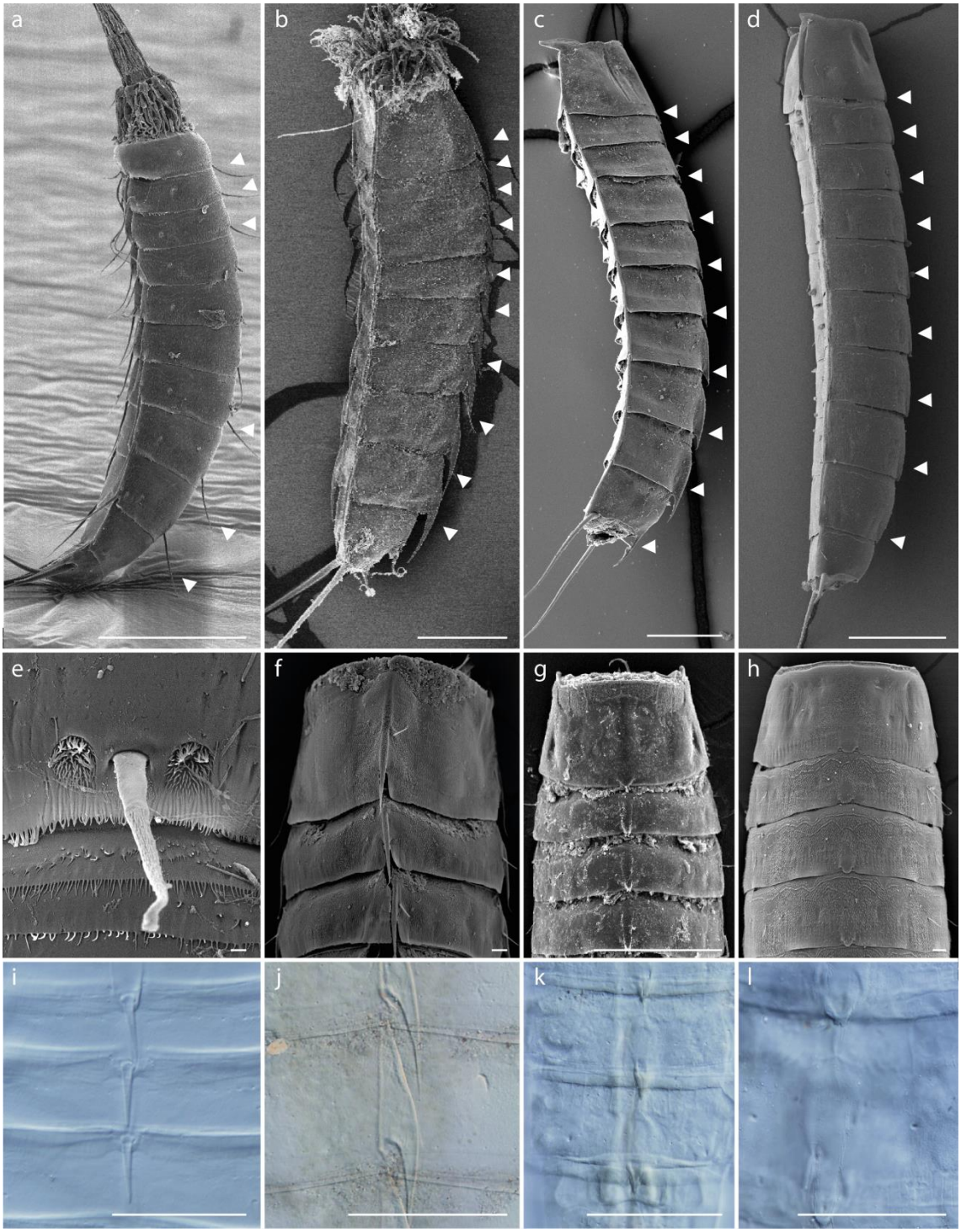
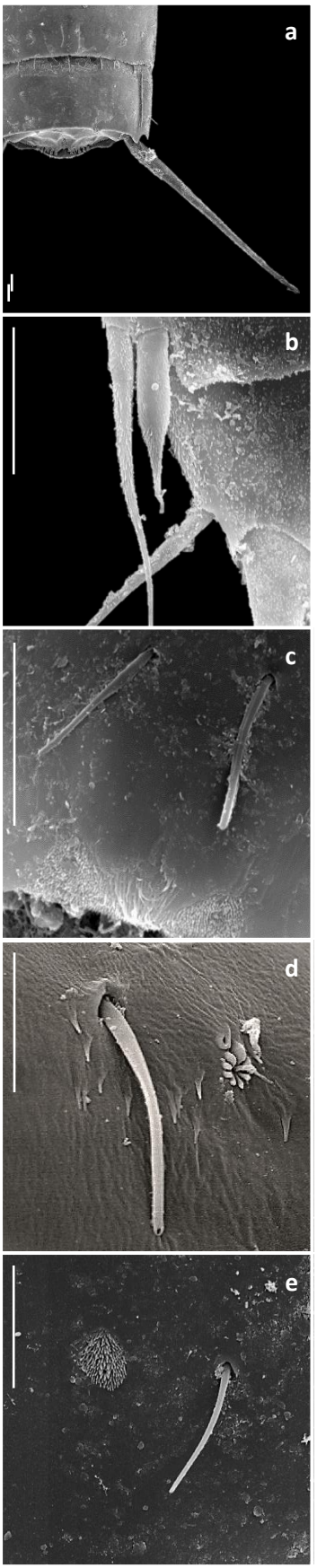


Figure 6. SEM and LM photographs on morphology of Kinorhyncha focused on the middorsal structure specializations; a, *Campyloderes*, lateral overview, showing middorsal spines (scale bar: 100 µm); b, *Mixtophyes abyssalis*, lateral overview, showing middorsal spinose processes (scale bar: 100 µm); C, *Pycnophyes cristatus*, lateral overview, showing keel-like middorsal processes (scale bar: 400 µm); d, *Pycnophyes frequens*, lateral overview, showing middorsal elevations (scale bar: 100 µm); e, *Campyloderes*, dorsal view, showing middorsal spine (scale bar: 1 µm); f, *Paracentrophyes quadridentatus*, dorsal view of anterior segments, detail of middorsal spinose processes (scale bar: 10 µm); g, *Pycnophyes dentatus*, dorsal view of anterior segments, detail of middorsal processes of segment 1 (scale bar: 100 µm); h, *Kinorhynchus mainensis*, dorsal view of anterior segments, detail of middorsal elevations that no surpass the posterior margin (scale bar: 10 µm); i, *Centroderes bonnyae*, dorsal view, detail of middorsal spines (scale bar: 5’ µm); j, *Mixtophyes abyssalis*, dorsal view, detail of middorsal spinose processes surpassing the posterior margin of the segments (scale bar: 100 µm); k, *Pycnophyes dentatus*, dorsal view of posterior segments, detail of middorsal processes (scale bar: 100 µm); l, *Pycnophyes dentatus*, dorsal view of anterior segments, detail of middorsal elevations flanked by paradorsal setae (scale bar: 50 µm).



Tubes: elongated, flexible and tubular cuticular appendages of the trunk, showing generally two pieces: a proximal one, short and broader, articulated basally with the segment plate; and a distal one, slender and flanked by longitudinal flaps, ending in a terminal opening (Kristensen and Higgins, 1991; GªOrdóñez et al., 2000). Tubes might have a secretory function, and they have traditionally been referred to as adhesive tubes in older literature (Zelinka, 1928; Higgins, 1983; Kristensen and Higgins, 1991), but this assumption should be avoided until its actual function (secretory/adhesive or sensory) is confirmed. Tubes are commonly present in the trunk of cyclorhagids but not amongst the species of Allomalorhagida; however in the latter ones tubes frequently appear ventrally at segment 2 of males as a sexually dimorphic trait.

Setae: elongated cuticular appendages of the trunk, with a proximal basis articulated with the segment plate and a longer, flexible and tubular distal part, flanked by two lateral, flat rims and ending in a terminal opening (Fig. 7). Structurally they are a version of cyclorhagid tubes referred to above, only discernible with detail through SEM. These appendages are exclusive of most allomalorhagid genera.

1.2.1.2 *Sensory spots:* cuticular specializations for the reception of sensory stimuli. Even though sensory spots are present in both adults and juvenile stages (Neuhaus, 1993; Lemburg, 2002; Sørensen et al., 2010b), they are rarely reported in the old literature because the structure is hard to visualize with light microscopy (LM). Three types of sensory spots can be distinguished by the number and arrangement of cuticular papillae, position and differentiation of pores. Generally, sensory spots consisting of a little round to oval or drop like area with many micropapillae (up to 100), bearing several pores and cilia (Fig. 7). The type 1 has a central and a lateral pore which open at the same level (Merriman and Convin, 1973; Higgins, 1983; Nebelsick, 1992), whereas the opening of the lateral pore in the type 2 is elevated at the tip of a short cuticular tube (Brown and Higgins, 1983; Higgins andKristensen, 1988; Nebelsick, 1992).

Figure 7. SEM and photographs on morphology of Kinorhyncha. a, *Pycnophyes pardosi,* detail of lateral terminal spine (scale bar: 10 µm); b, *Semnoderes armiger*, detail of cuspidate and acicular spines (scale bar: 20 µm); c, *Pycnophyes dolichurus*, detail of middorsal elevation flanked by paradorsal sensory spots and setae (scale bar: 10 µm); d, *Pycnophyes lageria*, detail of seta and *Nanaloricus*-like sensory spot (scale bar: 10 µm); e, *Pycnophyes pardosi*, detail of seta and a typical sensory spot with two pores surrounded by a high number of papillae (scale bar: 10 µm).

The type 3 is located at the terminal trunk segment and it consists of a conus-like with few cuticular papillae on its tip (Brown, 1985; Nebelsick, 1992). A few number of species, both cyclorhagids and allomalorhagids, possess a special kind of type 1 sensory spots, commonly referred to as flosculi or *Nanaloricus*-flosculi (N-flosculi), with a reduced number of cuticular papillae (8-10), arranged in a single circle and surrounding a central pore (Fig. 7).

1.2.1.3. Glandular cell outlets: epidermal glandular cells produce a mucous-like substance that emerges through small cuticular perforations or large gland cell outlets (Fig. 8). Two types of glandular cell outlets have been described (GªOrdóñez et al., 2000) and they play a special taxonomic role for some cyclorhagid genera. Type 1 gland cell are large, paired and located in the anterior half of some segments, in lateroventral, laterodorsal or subdorsal position, and show an oval shape in cross-section filled by a high amount of secretory product. Type 2 gland cell is smaller to the first type, and shows different shapes, and it occurs on both dorsal (paired or unpaired) and ventral position (paired) in the anterior part of the segment, close to the pachycyclus and the area of thinnest cuticle. The *cuticular scars* refer to a special kind of surface openings of internal glands. They are arranged segmentally in the dorsal and ventral sides in most kinorhynchs, with a major taxonomical relevance in Allomalorhagida. Cuticular scars may appear as scattered dot-shaped, rounded-oval-shaped, as dotted lines or groove-shaped (Fig. 8). This character is usually conspicuous under LM observation, but it is not detectable with SEM.

1.2.1.4. Cuticular hairs: small filiform cuticular projections that usually arise from the surface through a perforation site (Fig. 8). Both their shape (elongated, bracteate, leaf-like or scale-shaped) as well as their distribution pattern have taxonomical relevance in some genera, mostly in cyclorhagids.

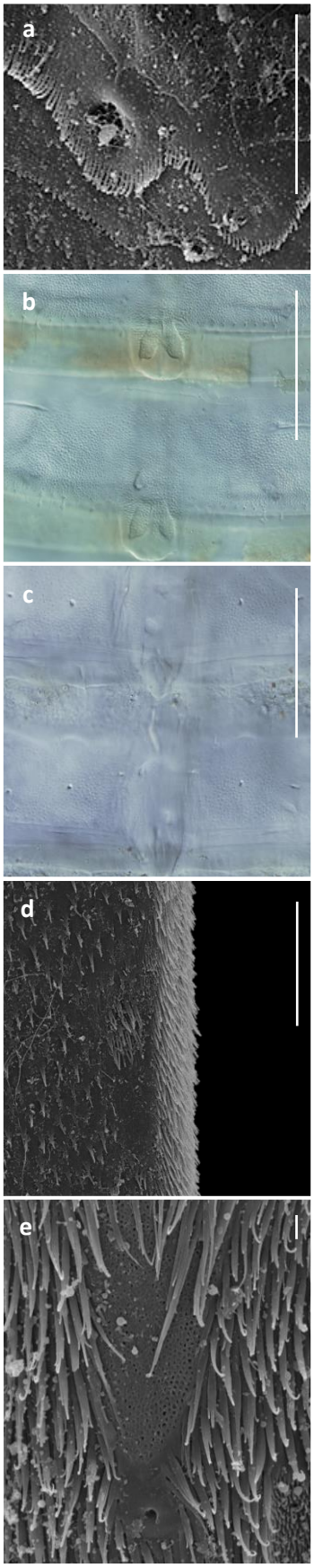


Figure 8. SEM and LM photographs on morphology of Kinorhyncha. a, *Kinorhynchus* sp., detail of glandular cell outlet (scale bar: 10 µm); b, *Pycnophyes aulacodes*, detail of dorsal groove-shaped cuticular scars (scale bar: 50 µm); c, *Pycnophyes dentatus*, detail of dorsal scattered dot-shaped cuticular scars (scale bar: 50 µm); d, *Pycnophyes norenburgi*, cuticular hairs and protonephridial opening as tubular set (scale bar: 10 µm); e, *Echinoderes coulli*, cuticular hairs and a detail of protonephridial opening as sieve plate (scale bar: 1 µm), courtesy of M.V. Sørensen.

1.2.1.5. Protonephridial openings: the paired protonephridia open to the outside on segment 9 by a system of lateral clustered perforations. The structure appears either as a cribate plate (sieve plate) in cyclorhagids or as a tubular set in allomalorhagids (Fig. 8), being its observation in the latter ones more difficult under LM.

1.2.2. Internal morphology

In addition to the studies focused on the taxonomy of the phylum, several researches were performed to improve our knowledge on its internal anatomy. The gross internal anatomy of kinorhynchs was firstly revealed in the Reinhard monograph (1885, 1887). Few years later, the monograph published by Karl Zelinka at the beginning of the XX century showed the general outline of the internal organization of kinorhynchs, with an amazing amount of details (Zelinka, 1928). Some other researchers contributed with isolated observations (Nyholm, 1947) but it was not until the end of the XX century when new observations with Transmission Electron Microscopy (TEM), mainly made by Adrianov, Higgins, Neuhaus and co-workers (Adrianov et al., 1989, 1990; Brown, 1989; Adrianov and Malakhov, 1990, 1991, 1994, 1999b; Neuhaus, 1988, 1991, 1994, 1997; Kristensen and Higgins, 1991; Nebelsick, 1992a-b, 1993; Neuhaus and Higgins, 2002) improved significantly the knowledge of the issue. In recent years, Confocal Laser Scanning Microscopy (CLSM) has been applied to the study of kinorhynchs, focused on the muscular and nervous systems (Müller and Schmidt-Rhaesa, 2003; Rothe and Schmidt-Rhaesa, 2004; Schmidt-Rhaesa and Rothe, 2006; Herranz et al., 2013, 2014a). Kinorhynchs lack both circulatory and respiratory systems, and the circulation and gas exchange is by diffusion (Neuhaus, 2013).

1.2.2.1. Cuticle and epidermis: Initially, the epidermis was assumed to be syncytial but its arrangement by individual, non-ciliated cells was confirmed through TEM (Moritz and Storch, 1972a; Kristensen and Higgins, 1991; Neuhaus 1991, 1994; Adrianov and Malakhov, 1994). The epidermis produces the cuticle, which is formed by a chitinous basal layer and a monolamellar, membrane-like epicuticle. In addition, a fibrillar layer at the articulation areas of tergal and sternal plates as well as between neighboring segments is present. The cuticle extends along the surface of the whole body, covering trunk, neck, introvert, sensory spots, tubules, gland cells, protonephridial openings and over a large portion of the alimentary canal, including the mouth (located at the mouth cone), foregut and hindgut (Boykin, 1965; Adrianov et al., 1990; Neuhaus, 1993; Adrianov and Malakhov, 1994; GªOrdóñez et al., 2000). In every segment, the cuticle is thicker at the anterior area, protruding towards the interior of segments 2-11, forming the pachycycli for muscle attachment (Fig. 9). The species of kinorhynchs with thick cuticle may develop two conspicuous kind of cuticular structures (Kristensen and Higgins, 1991). First, the tergal and sternal plates may be joined by a special

tergosternal articulation, the *ball and socket joint*, consisting of a sternal cuticular ring and a tergal spherical process. Second, internal cuticular thickenings may occur along the midventral joint, reaching their maximum development at the posterior segments, forming the so called *Mittelwülste* or just *apodemes*.

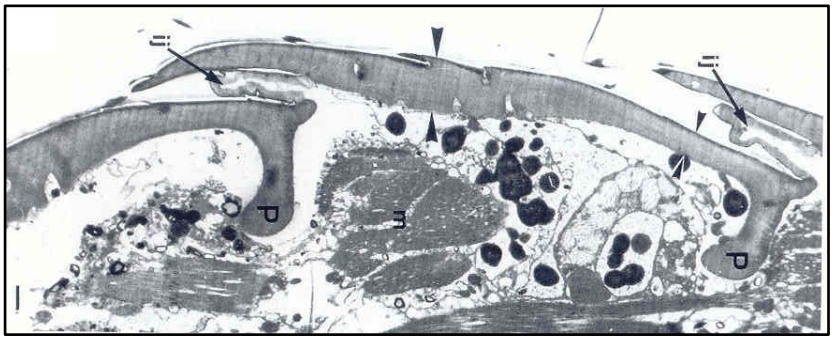


Figure 9. TEM picture of *Echinoderes cantabricus.* Dorsal side of the trunk on sagittal section, showing a complete segment. Abbreviations: P, Pachycyclus; ij, the folded intersegmentary joints, m, muscle (Scale bar: 1 mm). From GªOrdoñez et al. (2000).

1.2.2.2. Muscular system: The trunk has one pair of longitudinal ventral and dorsal muscles between subsequent pachycycli. These muscles have been described as segmentary, but some muscles of adjacent segments may meet at the same pachycyclus or may even extend over the pachycyclus elongating through two or several segments (Schmidt-Rhaesa and Rothe, 2006; Herranz et al., 2014a). Just diagonal -between two adjacent segments- and dorsoventral muscles -attached to the tergal and sternal plates of the same segment- are segmentally arranged. Several pairs of muscles may be identified at the posteriormost segment, which are involved in movements of the male penile spines, the gonopores in females and the terminal and lateral terminal spines. The placids of the neck are connected by circular muscles. Feeding and locomotion take place by movement of eversion and retraction of the introvert, which is characterized by a complex net of longitudinal and circular muscles: oral style muscles, circular muscles at the base of the scalids, introvert and pharynx retractors, mouth cone retractors and pharynx protractors. Moreover, circular and longitudinal muscles are located in the pharyngeal bulb (with anterior and posterior pharyngeal sphincters) and in the midgut, as well as paired dilatator muscles at the hindgut are present.

1.2.2.3. Digestive system: TEM and CLSM investigations were made in species of both Allomalorhagida and Cyclorhagida (Merriman and Corwin, 1973; Nyholm and Nyholm, 1976; Brown, 1989; Neuhaus, 1991, 1994; Nebelsick, 1993; Adrianov and Malakhov, 1990, 1994; Kristensen and Higgins, 1991; Müller and Schmidt-Rhaesa, 2003; Rothe and Schmidt-Rhaesa, 2004; Schmidt-Rhaesa and Rothe, 2006). The alimentary canal is straight and begins in the mouth cone, where the mouth is located, and ends in the anus. The whole system is cellular and composed of the foregut (including mouth and mouth cone, pharyngeal crown, pharynx, short esophagus), the non-ciliated midgut and the hindgut (rectum and anus). Foregut and hindgut are lined by cuticle, which shed at molting.

1.2.2.4. Nervous system: Although few, some studies exist on both Allomalorhagida and Cyclorhagida applying TEM and CLSM techniques (Kristensen and Higgins, 1991; Nebelsick, 1993; Neuhaus and Higgins, 2002; Herranz et al., 2013). Kinorhynchs share an intraepithelial nervous system that consists of a three-lobed, circumenteric brain from which 8, 10 or 12 longitudinal nerves arise. These fuse into five cords (two subdorsal, two ventrolateral and one midventral), connected by two commissures per trunk segment, plus a foregut nervous system (Nebelsick, 1993; Herranz et al., 2013). The longitudinal nerves innervate the introvert, neck, and trunk, and only occasionally show perikarya in posterior segments (Zelinka, 1928; Kristensen and Higgins, 1991; Nebelsick, 1993; Neuhaus and Higgins, 2002; Herranz et al., 2013). The ring-like brain surrounds the anterior part of the gut and the introvert retractor muscles and is divided into three regions: the anterior (forebrain) and posterior (hindbrain) neuronal somata separated by a central neuropil (midbrain). The forebrain is a ventrally open ring organized into a 10-lobed structure, whereas the midbrain is a closed ring with a neuropil and the hidbrain presents numerous perikarya arranged in irregular clusters (Kristensen and Higgins, 1991; Nebelsick, 1993; Neuhaus, 1994; Neuhaus and Higgins, 2002). Recent research on the cyclorhagid nervous system using CLSM (Herranz et al., 2013) confirmed these observations and showed that the terminal end of the ventral cord seems to be associated with the terminal spines. Unfortunately, this last investigation was centered on Cyclorhagida, and therefore only assumptions can be made regarding the Allomalorhagida.

1.2.2.5. Sensory system: numerous structures are involved in the reception of stimuli, both in the introvert, epidermal and inner organs. Several investigations through TEM showed that the appendages of both the introvert and the mouth cone, including scalids, inner and outer oral styles, have a sensory function. These appendages contain ciliary sensory cells that may either connect with the surface through a pore, with a supposed chemoreceptive function (for scalids, inner and outer oral styles) (Moritz and Storch, 1972a, Brown, 1989; Adrianov and Malakhov, 1990; Kristensen and Higgins, 1991; Nebelsick, 1993; Neuhaus, 1994) or without connection to the outside with a supposed mechanoreceptive function (trichoscalids) (Moritz and Storch, 1972b; Kristensen and Higgins, 1991). In addition, the presence of subcuticular photoreceptors with red pigment and a lens has been reported for some species of Cyclorhagida (Zelinka, 1928; Sørensen, 2006) whereas those of Allomalorhagida lack pigmentation (Brown, 1985; Kristensen and Higgins, 1991; Neuhaus, 1997). As for the epidermal sensory system, it is formed by the sensory spots. The ultrastructure of these receptors mainly consists of monociliary cells that connect to the surface through subcuticular tubes, and therefore a chemoreceptive function is assumed (Brown, 1989; Adrianov and Malakhov, 1990; Adrianov et al., 1989; 1990; Kristensen and Higgins, 1991; Neuhaus et al. 2013).

Lastly, the inner receptor organs are connected to the digestive and excretory systems. The sensory cells associated with the midgut are autonomous, independent of the central nervous system, and have a likely sensorimotive function. This kind of sensory cells may be involved in the activation of peristaltic gut movements as well as in the defecation processes (Neuhaus, 1991, 1994; Neuhaus and Higgins, 2002). Something similar may occur with the protonephridium sensory cells, which are supposedly involved in the control of the filtering process (Neuhaus, 1988).

1.2.2.6. Excretory system: a pair of ciliated, club-shaped, elongate protonephridia are present in segment 8 in laterodorsal position. Nephridial tubes open to the outside laterally in segment 9 through a sieve plate with 10 to numerous pores (Adrianov and Malakhov, 1994; Pardos et al., 1998; Neuhaus and Blasche, 2006; Sørensen et al., 2009; Neuhaus et al., 2013). Alternatively, the opening consists of several pores or tubes partly covered by short cuticular hairs (Sánchez et al., 2014c, Chapter I).

1.2.2.7. Reproductive system: Kinorhynchs are dioecius with a sexual reproduction and external sexual dimorphism (Zelinka, 1928; Higgins, 1974). Both sexes have paired saccate gonads (Zelinka, 1928; Higgins, 1974) that open to the outside through a pair of gonopores ventrally located between segments 10-11 (Higgins, 1969a, 1983, 1990). In females, the oviduct forms a diverticulum near the gonopore, named seminal receptacle, where the sperm stay retained (Zelinka, 1928; Higgins, 1974; Brown, 1983; Kristensen and Higgins, 1991; Adrianov and Malakhov, 1999b; Neuhaus, 1999; Neuhaus and Sørensen, 2012; Neuhaus et al., 2013). Regarding the external sexual dimorphism, it is easily detectable mostly by the presence of penile spines in males and gonopores in females. Additional sexual characters are the paired large ventral tubes on segment 2 in many males of Allomalorhagida, the presence of lateral terminal accessory spines in females of most Cyclorhagida and the dorsal spines on segment 10, often crenulated, and papillae (Zelinka, 1928; Higgins, 1974; Kristensen and Higgins, 1991; Adrianov and Malakhov, 1999b).

1.3. REPRODUCTION AND DEVELOPMENT

The fertilization is internal and seems to take place by spermatophore transfer, although the available observations are very limited. The spermatophores produced by males through an unknown mechanism are supposedly picked up by the females during the copula (Neuhaus, 1999; Neuhaus and Higgins, 2002). Then, the sperm arranged as a tangled ball starts the migration into the female's body through the gonopores (Brown, 1983) (Fig. 10). It is well known that the sperm may be stored inside the seminal receptacle of females (Zelinka, 1928; Kristensen and Higgins, 1991; Adrianov and Malakhov 1994, 1999b; Neuhaus and Higgins, 2002). Fertilization has never been

observed, but lastly, the fertilized egg is deposited at the sediment (Higgins, 1974; Kozloff, 1972, 2007). However, the knowledge about these spermatophores and their function in reproduction is still limited, as well as how males produce the spermatophores, how these are transferred from males to females, how and where the fertilization of the oocytes occurs and why the females store the sperm (Neuhaus, 1999).

Also, the role of the penile spines of males and additional structures supposed involved in the reproduction has to be determined, as well as the female oviposition (Nyholm, 1947; Lang, 1963; Brown, 1983; Kristensen and Higgins, 1991; Neuhaus and Higgins, 2002). Timing or seasonality of reproduction, together with the variation in reproductive biology among the different kinorhynch taxa are still unexplored research fields.

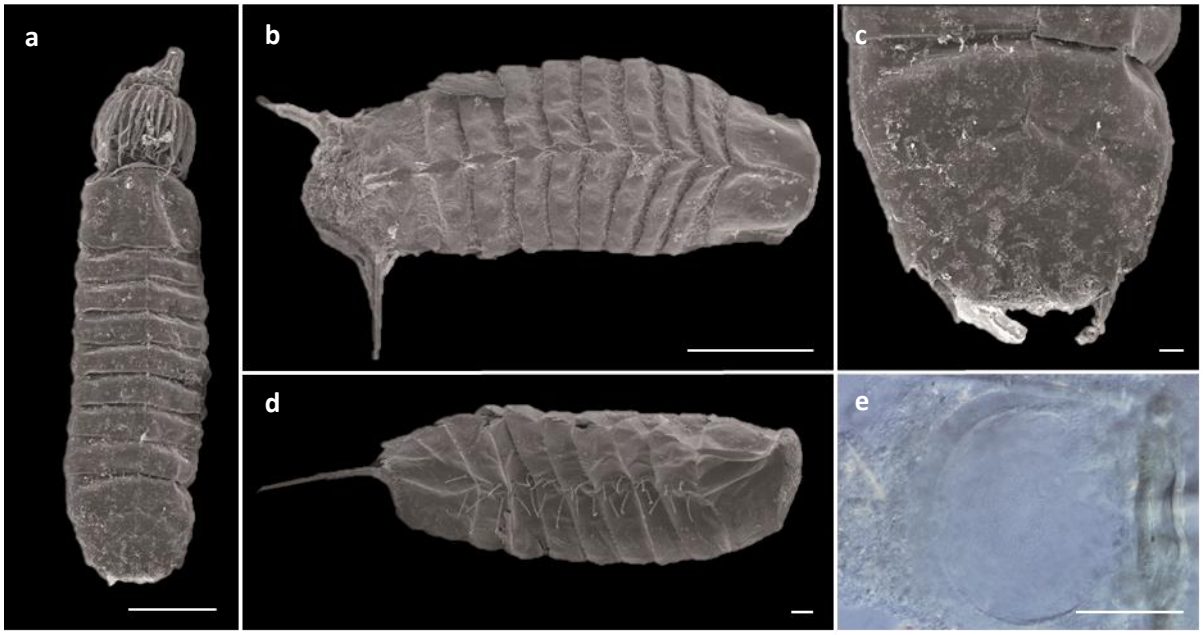


Figure 10. SEM pictures of juvenile stages of Pycnophyidae; a, ventral overview (scale bar: 100 µm); b, dorsal overview (scale bars: 100 µm); c, detail of the terminal anlagen of the three to four last segments (scale bar: 10 µm); d, ventral overview (scale bar: 10 µm); e, LM picture of a spermatophore attached to the posterior segment of a female of *Pycnophyes norenburgi* (scale bar: 50 µm).

Kinorhynchs have a direct development, with no larval or dispersal stage. The embryo contained into the egg enveloping does not look externally segmented and does not bear any kind of spine or cuticular appendages. Then, it elongates and the introvert with early scalids as well as the initial segmentation of the trunk becomes visible (Kozloff, 1972). The hatching of the egg occurs about 10 days after the ovoposition (Kozloff, 1972, 2007) and the first juvenile stage emerges. This first free-living juvenile consists of the introvert with scalids, the placids of the neck and at least eight trunk segments undivided into plates. The terminal trunk region is formed by an anlagen of the three to four last segments (Fig. 10). The adult stage is reached through six juvenile stages, each of them derived from subsequent molts (Zelinka, 1928; Brown, 1985; Neuhaus, 1993, 1995; Sørensen et al., 2010b) (Fig. 10). It is supposed that the molt process is induced by the hormone ecdysone, but it has

never been proved. When the new stage is fully developed, the specimen sheds the exuvia withdrawing its introvert and emerging from the anterior end of the old cuticle (Neuhaus, 2013). The animal molts the whole cuticle during each hatching, including the part of the cuticle-lined digestive system, that is, the foregut and hindgut (Zelinka, 1928; Neuhaus 1993, 1995; Neuhaus and Sørensen, 2012).

1.4. GEOGRAPHICAL DISTRIBUTION

Since the discovery of the phylum studies on kinorhynchs have been mostly focused on taxonomical and morphological contributions, all of them on individuals obtained from punctual gatherings in different localities around the world (Fig. 11). Extensive sampling campaigns were performed only along the American East Coast, Caribbean Sea, North Pacific Coast of Russia, European West Coast (Higgins, 1960, 1961, 1964a, 1964b, 1965, 1966a, 1966b, 1967, 1968, 1969a, 1969b, 1977a, 1977b, 1978, 1983, 1985, 1986a, 1986b, 1990; Nebelsick, 1990; Sørensen et al., 2005, 2007, 2012; Sørensen, 2007; Adrianov and Malakhov, 1999a; Landers et al., 2012; Herranz et al., 2014b; Sørensen and Landers, 2014) and around the Iberian, the Korean and the Italian Peninsulas (Zelinka, 1928; Sørensen and Rho, 2009; Sørensen et al., 2010a, 2010b, 2010c, 2010d, 2012a, 2012b, 2013; Lundbye et al., 2011; Herranz et al., 2012; Sánchez et al., 2012; Thomsen et al., 2013; Altenburger et al., 2015). Hence, the kinorhynch diversity in these areas can be considered relatively well-known, even though new surveys often yield the discovery of new species.

Figure 11. Worldwide records of kinorhynchs. Green dots mark kinorhynchs identified and reported in the literature. Red dots mark areas where kinorhynchs have been collected but still unidentified.

As for the Iberian Peninsula, the knowledge of the kinorhynch diversity was quite low until the biogeographical study made by Sánchez et al. (2012). The Iberian Peninsula is an interesting area for the study of kinorhynchs due to its singular location in between the Atlantic Ocean and the Mediterranean Sea, providing an ideal situation for biogeographical and ecological studies. This contribution compiled results from 21 years of samplings in different areas along the Iberian Peninsula coasts. Around 2000 specimens were checked, with a total of 29 species identified accommodated in 11 genera (Fig. 12), most of them new records for the Iberian Peninsula. Besides data on the diversity and biogeography, this work gave ecological information related to depth, sediment and abundance, showing that the distribution of some kinorhynchs species seems to be highly related to certain types of sediments and depth. Our results showed that several kinorhynch species had a wide distribution, present in both Mediterranean and Atlantic localities, such as *Pycnophyes dentatus* (newly recorded for the Iberian Peninsula), *Echinoderes cantabricus*, *E. hispanicus* and *E. dujardinii*. Specifically, *Pycnophyes dentatus* was the most ubiquitous species in the samples, present at almost all localities and in high number. Oppositely, other species seem to have restricted distributions, sometimes found in a single or very few localities (Fig. 12).

At both global and local levels, the current knowledge of the geographical distribution of kinorhynchs corresponds better to sampling distribution chosen by the scientist than to real taxa distribution, which evidences that the diversity and biogeography of kinorhynchs is largely biased and still far away from being known (Sánchez et al., 2012, 2013; Neuhaus, 2013).

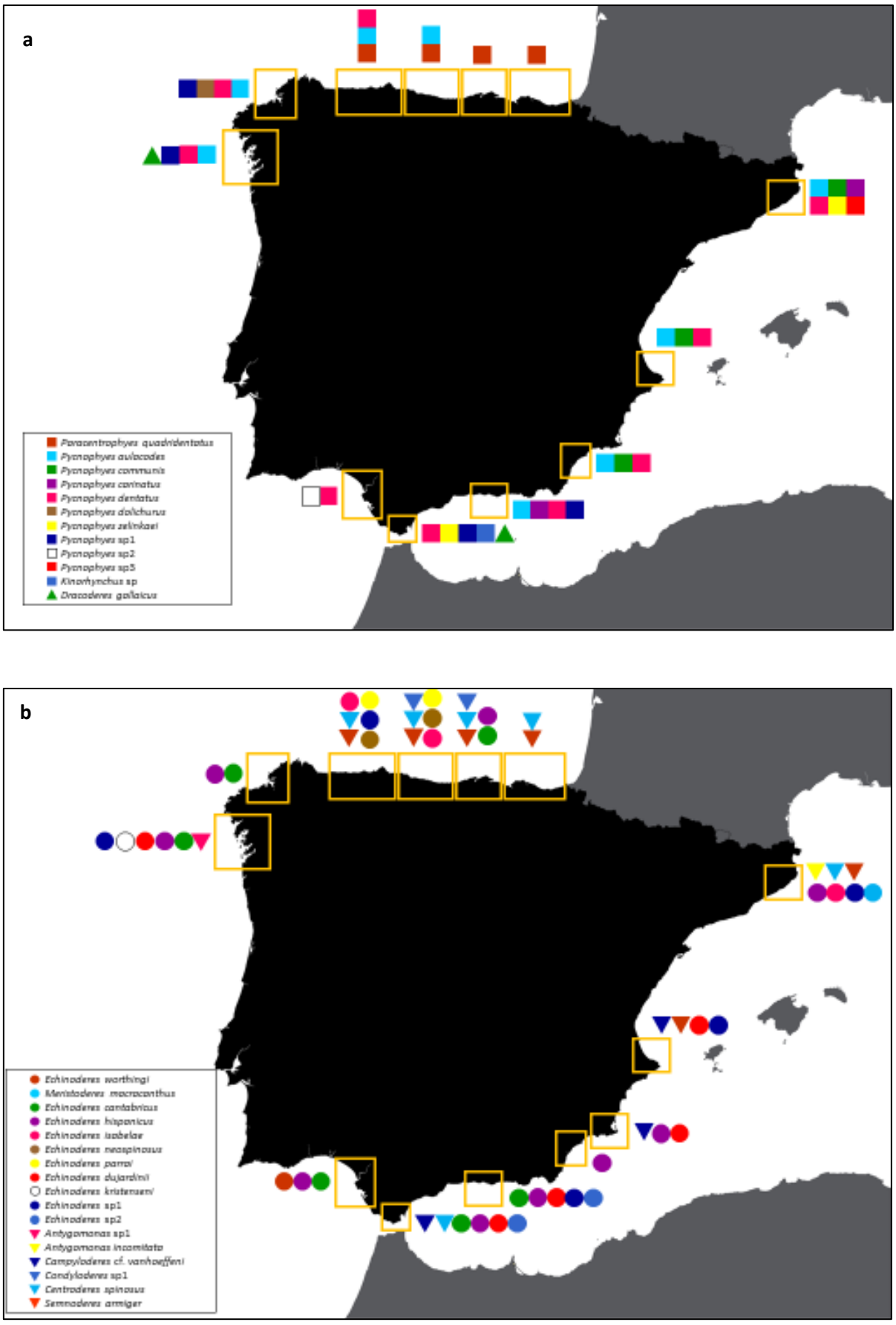


Figure 12. Kinorhynch species distribution along the coast of the Iberian Peninsula, according to Sánchez et al. (2011). a, Allomalorhagida species; b, Cyclorhagida species.

1.5. SYSTEMATICS AND PHYLOGENY

Kinorhyncha has been classically considered as closely related to priapulids and then to loriciferans after its discovery. Such relationship, Kinorhyncha-Priapulida-Loricifera, together with Nematoda and Nematomorpha, was proposed by Adrianov (Adranov and Malakhov, 1995, 1999a), who considered each of these taxa as a class and grouped into the "Cephalorhyncha", which was accommodated into the phylum "Aschelminthes" (See, for example, Hyman, 1951). However, the phylum Aschelminthes resulted polyphyletic under general metazoan phylogenetic analyses. Subsequently, the phylum was rejected and each of its classes was promoted to the phylum level. Even though most of their taxa are no longer considered closely related, some of the relationships still remain: Kinorhyncha, Priapulida and Loricifera are now grouped into the monophyletic Scalidophora (with introvert bearing scalids, see Lemburg, 1995), while Nematoda and Nematomorpha are grouped into the Nematoida; and all together form the Cycloneuralia (Schmidt-Rhaesa, 2007; Nielsen, 2012) (Figs. 13, 14). The group is well defined by morphological apomorphies, such us the presence of a collar-shaped peripharyngeal brain (Ahlrichs, 1995; Nielsen, 2012), but its monophyly is hardly ever yielded in molecular analyses (Garey, 2001; Telford et al., 2008; Dunn et al., 2008; Budd and Telford, 2009; Hejnol et al., 2009; Edgecombe et al., 2011). Currently, Cycloneuralia is related to the Panartropoda, both constituting the Ecdysozoa. The Ecdysozoa monophyly is supported by molecular (Dunn et al., 2008, 2014; Hejnol et al., 2009; Paps et al., 2009; Pick et al. 2010) (Fig. 14) and morphological data (Fig. 13), mostly by the presence of non-ciliated cuticle that sheds periodically through apparently ecdysteroid hormones control (Aguinaldo et al., 1997; Nielsen, 2012).

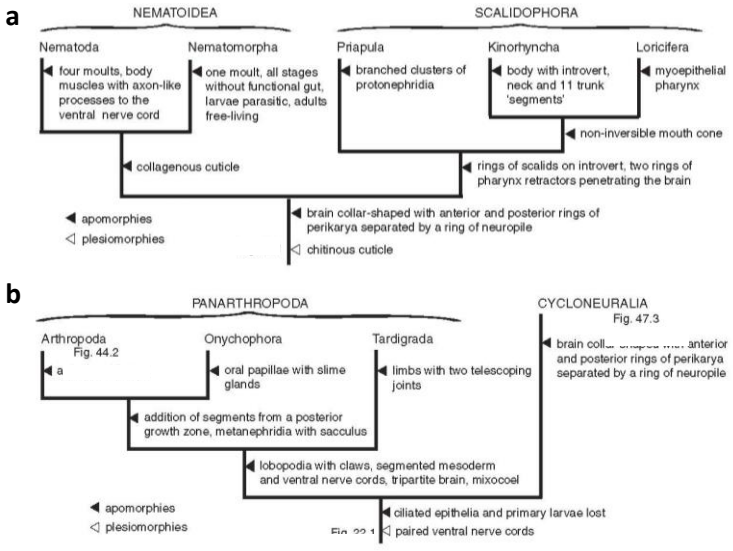


Figure 13. Phylogeny of a, Cycloneuralia; b, Ecdysozoa showing apomorphies and plesiomorphies for each group. From Nielsen, 2012, "Animal Evolution. Interrelationship of the living phyla".

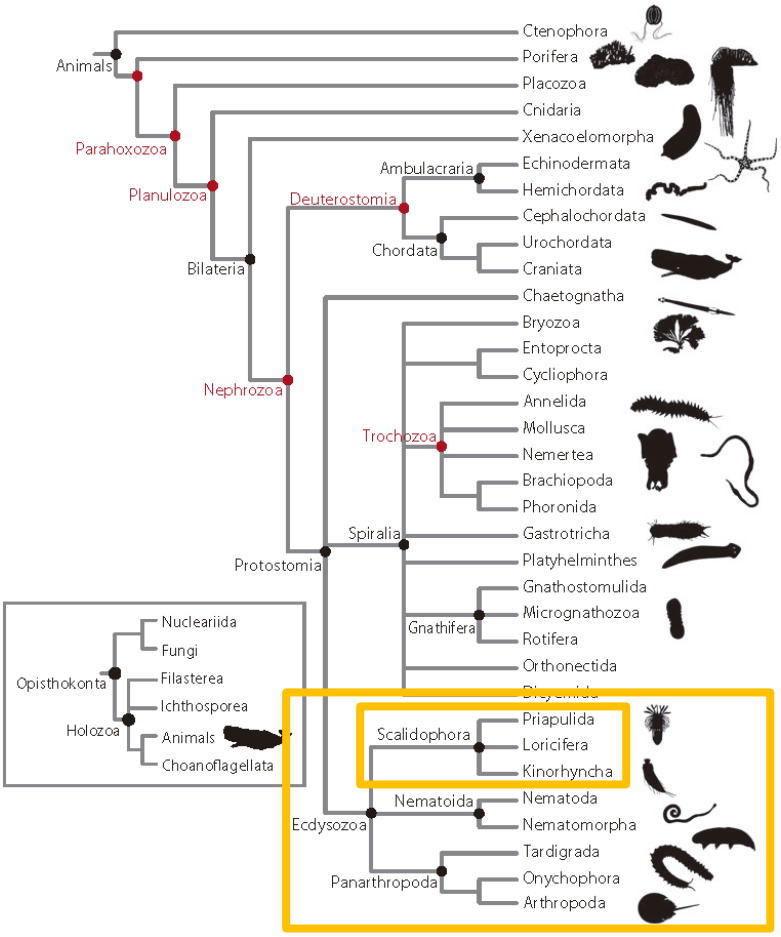


Figure 14. A hypothesis of animal phylogeny, compiled across multiple studies. Black dots denote clades that havebroad consensus across studies. Red dots denote clades that have poor or conflicting support or whose exact composition is uncertain. From Dunn et al., 2014.

Within the Kinorhyncha, phylogenetic relationships have been mistakenly influenced from the taxonomical classification of the phylum; however, the conceptual process should run in the opposite way, that is, a classification should be stated upon the branched tree of phylogeny. We realize now that all traditional classifications were founded on a very comprehensive knowledge of kinorhynch diversity and morphology through the experience of many years of research by a few authors. Unfortunately, such classifications did not reflect the evolution of the Kinorhyncha, but were absolutely phenetic and based on the presence of shared morphological characters and not on empirical analysis. Conceptually, the weakness of these classifications is that they did not follow the Hennigian thinking and procedures, and hence they do not discriminate between apomorphic and plesiomorphic character conditions.

The traditional Kinorhyncha classification was based on systematic hierarchies stablished by Zelinka (1907) and later modified and updated by Higgins (1964b, 1990) and Adrianov and Malakhov (1996, 1999a), all of them following essentially the same system with some minor differences. At

high taxonomical levels, the taxa were established upon the arrangement of segmental plates, especially those of segments 1 and 2, a feature directly related to the trunk closing mechanism. Under this idea, all classifications agreed to divide the phylum Kinorhyncha into two main groups: Cyclorhagida and Homalorhagida. Within the Cyclorhagida, the species appeared grouped into three clusters according to the closing system: Conchorhagae (closing bilaterally, shell-like), Critptorhagae (radially closing system, without placids) and Cyclorhagae (radially closing system, with placids). Along the XX century, new forms of kinorhynchs were discovered, sometimes with bizarre morphological features, such are the genera *Zelinkaderes*, *Cateria*, *Antygomonas*, *Neocentrophyes*, *Paracentrophyes* or *Dracoderes*. New taxa were accordingly accommodated into the system at more or less forced positions even at the family level when needed (Sørensen and Pardos, 2008). At the end of the century, the classification widely accepted was that pictured in Figure 15.

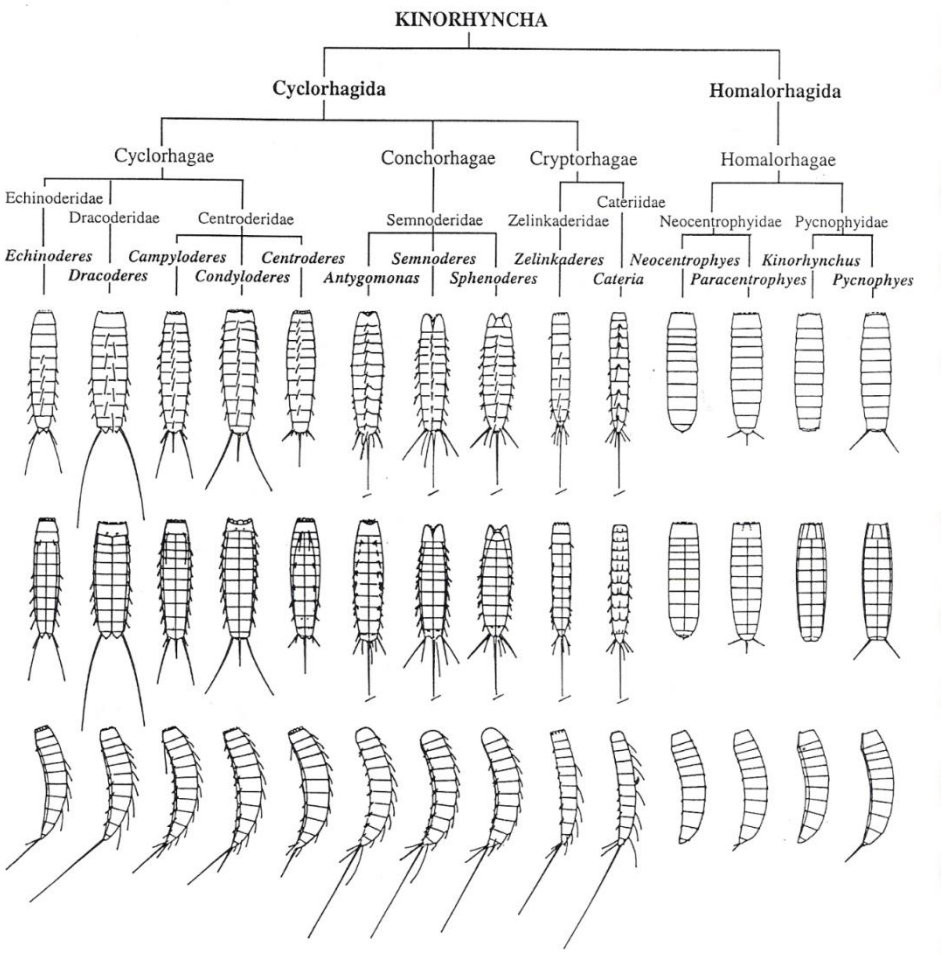


Figure 15. Diagram showing the traditional classification of the phylum prior to the molecular and morphological phylogenetic studies. This classification divides the phylum in two orders: Cyclorhagida and Homalorhagida (with four suborders: Conchorhagae, Critptorhagae, Cyclorhagae and Homalorhagae). Courtesy of R.P. Higgins.

Recently, the discovery and description of several new genera, some of them also with striking features, prompted an updated classification of the phylum still following the previous systems (Sørensen, 2013), that can be summarized as follows:

Phylum KINORHYNCHA Dujardin, 1851

Order Cyclorhagida Zelinka, 1896 (7 families)

Family Antygomonidae Adrianov & Malakhov, 1994 (1 genus)

Genus *Antygomonas* Nebelsick, 1990 (3 species)

Family Cateriidae Gerlach, 1956 (1 genus)

Genus *Cateria* Gerlach, 1956 (2 species)

Family Centroderidae Zelinka, 1896 (3 genera)

Genus *Campyloderes* Zelinka, 1913 (2 species)

Genus *Centroderes* Zelinka, 1907 (1 species)

Genus *Condyloderes* Higgins, 1969 (5 species)

Family Echinoderidae Bütschli, 1876 (5 genera)

Genus *Cephalorhyncha* Adrianov, 1999 (3 species)

Genus *Echinoderes* Claparède, 1863 (77 species)

Genus *Fissuroderes* Neuhaus & Blasche, 2006 (5 species)

Genus *Meristoderes* Herranz, Thormar, Benito, Sánchez & Pardos, 2012 (6 species)

Genus *Polacanthoderes* Sørensen, 2008 (1 species)

Family Dracoderidae Higgins & Shirayama, 1990 (1 genus)

Genus *Dracoderes* Higgins & Shirayama, 1990 (4 species)

Family Semnoderidae Remane, 1936 (2 genera)

Genus *Semnoderes* Zelinka, 1907 (3 species)

Genus *Sphenoderes* Higgins, 1969 (2 species)

Family Zelinkaderidae Higgins, 1990 (2 genera)

Genus *Triodontoderes* Sørensen & Rho, 2009 (1 species)

Genus *Zelinkaderes* Higgins, 1990 (4 species)

Order Homalorhagida Zelinka, 1896 (2 families)

Family Neocentrophyidae Higgins, 1969 (2 genera)

Genus *Neocentrophyes* Higgins, 1969 (2 species)

Genus *Paracentrophyes* Higgins, 1983 (3 species)

Family Pycnophyidae Zelinka, 1896 (2 genera)

Genus *Kinorhynchus* Sheremetevskij, 1974 (19 species)

Genus *Pycnophyes* Zelinka, 1907 (51 species)

Genera *incertae sedis*

Genus *Franciscideres* Dal Zotto, De Domenico, Garaffoni & Sørensen, in press (1 species)

Genus *Tubulideres* Sørensen, Heiner, Ziemer & Neuhaus, 2007 (1 species)

Genus *Wollunquaderes* Sørensen & Thormar, 2010 (1 species)

During the last decade several specialists paid attention to the internal relationships of the phylum and joined forces to shed light over this aspect of the kinorhynch knowledge. A series of molecular phylogenetic analyses were performed based on ribosomal genes (18S rRNA and 28S rRNA) (Dal Zotto et al., 2013; Yamasaki et al., 2013). These analyses recovered two large clades roughly consistent with the traditional classification, Homalorhagida and Cyclorhagida. The exception was the position of the cyclorhagid genus *Dracoderes*, which together with the newly described genus *Franciscideres* and a yet undescribed genus were grouped with the homalorhagids, even though all of them share morphological traits considered as apomorphic for Cyclorhagida (Dal Zotto et al., 2013; Yamasaki et al., 2013). Furthermore, the polyphyly of Cyclorhagida suggested in these papers has been very recently corroborated after a combined analysis of morphological and molecular data, which forced a comprehensive revision of kinorhynch systematics (Sørensen et al., in press) to the family and genus level. Moreover, the analysis did not yield a monophyletic clade consisting of the traditional homalorhagid genera and hence Homalorhagida was rejected as a taxonomic and phylogenetic unit. Therefore, the phylum is now divided into two classes, Cyclorhagida Zelinka, 1896 and Allomalorhagida Sørensen et al., in press, with the latter accommodating the traditional homalorhagid genera together with the genera *Dracoderes*, *Franciscideres* and the a new undescribed genus (Sørensen et al., in press). The resulting phylogeny and subsequent taxonomic arrangement of hierarchical taxa are shown in Figure 16 and Table 1.

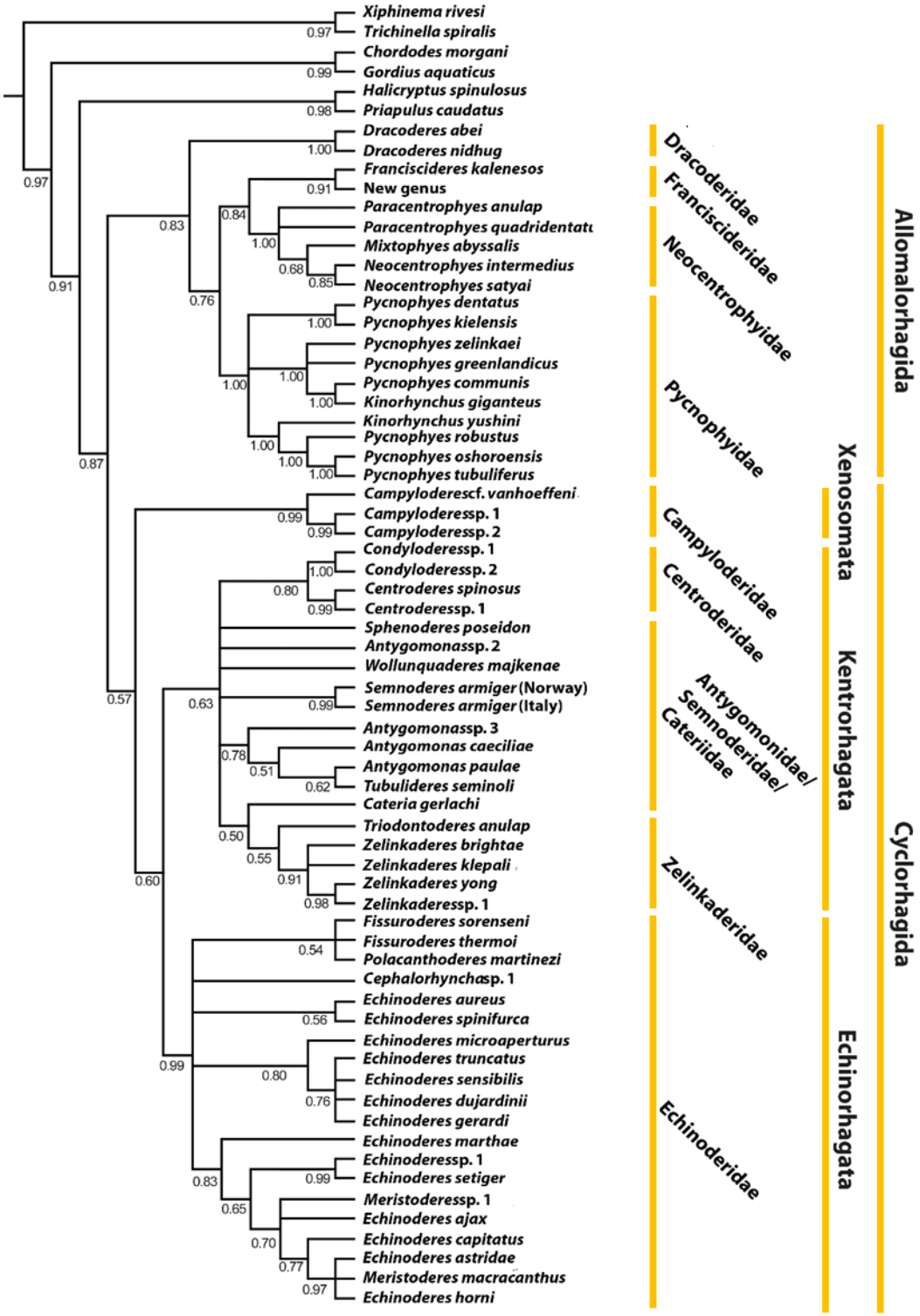


Fig. 16. Tree topology yielded by the Bayesian analysis of the combined morphological and molecular data sets. Numbers indicate posterior probabilities. Modified from Sørensen et al., in press.

Table 1. The new kinorhynch classification that includes correction of the taxonomic levels, assigning class rank to Cyclorhagida and Allomalorhagida, and consequently giving the order rank to the cyclorhagid groups Echinorhagata nom. nov., Kentrorhagata and Xenosomata. From Sørensen et al. (in press).

Class	Order	Family	Genus
Allomalorhagida nom. nov.	-	Dracoderidae Higgins & Shirayama, 1990	*Dracoderes* Higgins & Shirayama, 1990
	-	Franciscideridae Fam. nov.	*Franciscideres* Dal Zotto et al., 2013 New genus Yamasaki in prep.
	-	Pycnophyidae Zelinka, 1896	*Kinorhynchus* Sheremetevskij, 1974 *Pycnophyes* Zelinka, 1907
	-	Neocentrophyidae Higgins, 1983	*Mixtophyes* Sánchez et al., 2014 *Neocentrophyes* Higgins, 1969 *Paracentrophyes* Higgins, 1983
Cyclorhagida comb. nov.	Echinorhagata nom. nov.	Echinoderidae Zelinka, 1894	*Cephalorhyncha* Adrianov, 1999
			Echinoderes Claparède, 1863 *Fissuroderes* Neuhaus & Blasche, 2006 *Meristoderes* Herranz et al., 2012 *Polacanthoderes* Sørensen, 2008
	Kentrorhagata nom. nov.	Antygomonidae Adrianov & Malakhov, 1994	*Antygomonas* Nebelsick, 1990
		Cateriidae Gerlach, 1956	*Cateria* Gerlach, 1956
		Centroderidae Zelinka, 1869	*Centroderes* Zelinka, 1907 *Condyloderes* Higgins, 1969
		Semnoderidae Remane, 1929	*Semnoderes* Zelinka, 1907 *Sphenoderes* Higgins, 1969
		Zelinkaderidae Higgins, 1990	*Triodontoderes* Sørensen & Rho, 2009 *Zelinkaderes* Higgins, 1990
		incertae sedis incertae sedis	*Tubulideres* Sørensen et al., 2007 *Wollunquaderes* Sørensen & Thormar, 2010
	Xenosomata Zelinka, 1907	Campyloderidae Remane, 1929	*Campyloderes* Zelinka, 1907

This analysis clarifies most of the relationships amongst the major clades within Kinorhyncha, and establishes a new classification of the phylum following a natural systematic arrangement (Sørensen et al., in press). However, it led open up several questions regarding the internal relationships within some taxa, particularly those of the two largest kinorhynch families, that is, Echinoderidae Bütschli, 1876 and Pycnophyidae Zelinka, 1896. Moreover, the establishment of the new class Allomalorhagida (subject of this Thesis), which comprises the old homalorhagids plus other genera, all rearranged into a system of somehow uncertain relationships, deserves additional and detailed comments.

1.7. CLASS ALLOMALORHAGIDA

Since the erection of the Homalorhagida by Zelinka (1928) it suffered some modifications to the family and genus levels, even with the addition of a new family, Neocentrophyidae (Higgins, 1969). However, the monophyly of the Homalorhagida had never been questioned to date. The current classification of Kinorhynchs based on phylogeny proposed the erection of a new class, Allomalorhagida, which compiles all the genera of the traditionally named homalorhagids (*Pycnophyes*, *Kinorhynchus*, *Paracentrophyes*, *Neocentrophyes*, *Mixtophyes*) as well as *Dracoderes*, *Franciscideres* and a new, still undescribed genus, referred to in the following as New Genus (Sørensen et al., in press) (Figs. 16, 17; Table 1). This rearrangement was needed since the analyses consistently yielded Homalorhagida as paraphyletic or polyphyletic, with *Paracentrophyes*, *Mixtophyes* and *Neocentrophyes* grouping with New Genus and *Franciscideres* in most analyses, and *Pycnophyes* and *Kinorhynchus* grouping with *Dracoderes* in some of them. The phylogenetic results left Homalorhagida as a not natural phylogenetic group and hence it was rejected as a taxonomic and phylogenetic unit. As it was previously noted, the relationship among Dracoderidae, Franciscideridae, Pycnophyidae and Neocentrophyidae had not been proposed previously since Dracoderidae and Franciscideridae share several morphological traits considered as apomorphic for Cyclorhagida (Dal Zotto et al., 2013; Yamasaki et al., 2013).

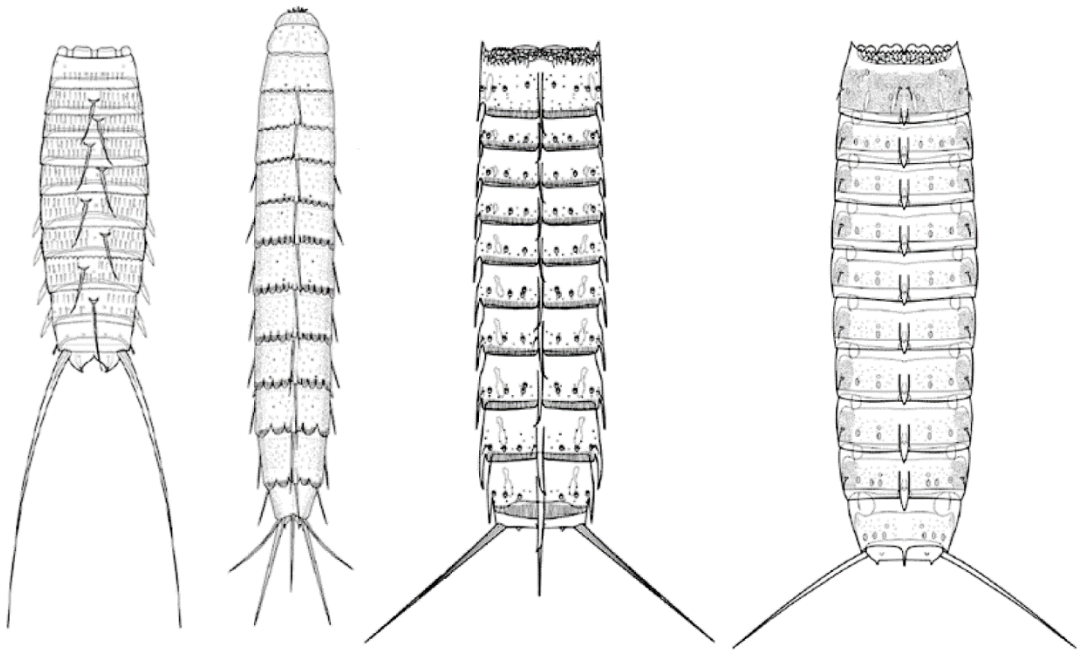
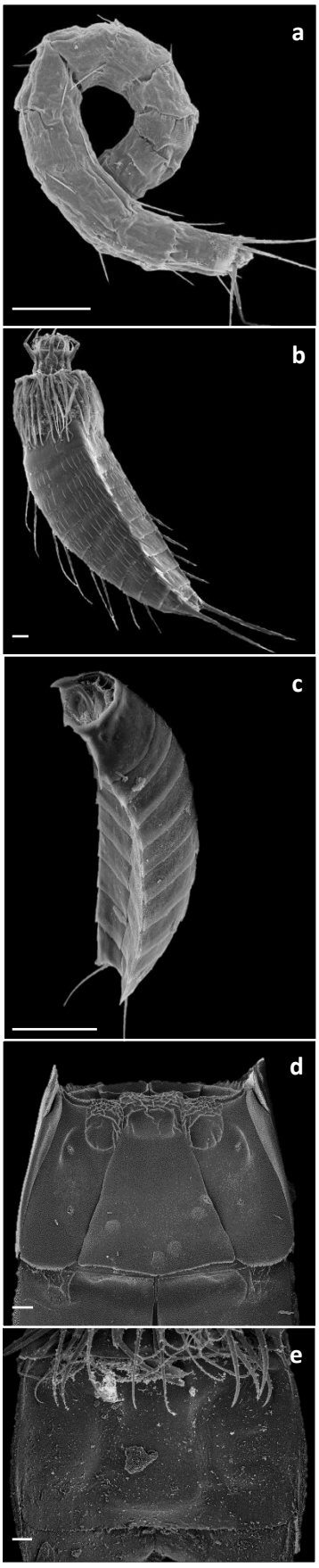


Figure 17. External morphotypes (dorsal views) of the Allomalorhagida families. a; Dracoderidae; b, Franciscideridae; c, Neocentrophyidae; d, Pycnophyidae.



The most important character by which Dracoderidae was originally accommodated into the cyclorhagids, other than its radially trunk closing system, was its trunk section, as heart-shape, its small size and the presence of spines along the trunk segments (dismissing the lateral terminal ones) (see Sørensen and Pardos, 2008). Neocentrophyidae and Pycnophyidae are generally bigger, with a nearly rectangular outline and a conspicuously triangular cross-section (Fig. 18). The species of Dracoderidae and Franciscideridae bear acicular spines on most of the segments, whereas the species of Neocentrophyidae have middorsal spines restricted to the last segments (10-11 in males, 11 in females), and species of Pycnophyidae does not bear spines (Fig. 18).

Besides the spines, additional morphological characters are useful in order to discriminate among the Allomalorhagida families and genera:

- *Segment 1*: it consists of one closed ring in the Dracoderidae and Franciscideridae, whereas it is divided into tergal and sternal plates in the remaining allomalorhagids. Among species of Neocentrophyidae the first trunk segment consists of one tergal and one sternal plate, with the ventral one being just partially divided anteriorly in *Paracentrophyes* or undivided in *Neocentrophyes* and *Mixtophyes*, whereas species of Pycnophyidae show one tergal and three sternal plates (Figs. 17, 18).

- *Segment 11*: the sternal region of the terminal trunk segment is divided into two plates in all Dracoderidae and Pycnophyidae species, whereas it is made up as a single, undivided plate in Neocentrophyidae, and the whole segment appears as a closed ring in Franciscideridae (Fig. 18).

- *Middorsal cuticular specializations*: the posterior margin of the tergal plates may either present some cuticular specializations or show a smooth margin, without any middorsal structure specialization (See Appendix 1). The shape of middorsal structures may be categorized into four different types (Fig. 6).

Figure 18. SEM photographs of selected Allomalorhagida species. a, *Franciscideres kalenesus*, lateral overview, showing ring shape of the trunk (scale bar: 50 µm), courtesy of M.V. Sørensen; b, *Dracoderes abei*, lateral overview, note the heart-shaped trunk (scale bar: 10 µm), courtesy of M.V. Sørensen; c, *Pycnophyes carinatus*, lateral overview, the trunk has the triangular shape typical of Pycnophyidae and Neocentrophyidae (scale bar: 100 µm); d, *Pycnophyes pardosi*, showing the sternal plate organization of segment 1 as totally divided in one midsternal and two episternal plates (scale bar: 10 µm); e, *Mixtophyes abyssalis*, showing the undivided sternal plate organization of segment 1 (scale bar: 10 µm).

Middorsal elevations are cuticular structures poorly developed, not protruding beyond the segment margin. *Middorsal processes* are protruding structures that surpass beyond the posterior margin of the segment and usually bear hairs along the middorsal line. These middorsal processes may be just a pointed protrusion of the posterior segment margin or have a conspicuous keel shape with a rigid terminal end. *Middorsal spinose processes* are non-articulated pointed projections of the posterior edge of the tergal plate. These have a conspicuous keel shape, with an elongate base and a flexible terminal end, beginning at the anterior third of the segment and surpassing half of the following segment. *Middorsal spines* are unpaired, cuspidate cuticular appendages of the trunk with an articulated proximal basis and a longer, rigid distal part, ending in a pointed, closed tip.

As it was previously referred, spines are present on several segments in all species of Dracoderidae and Franciscideridae, whereas the species of Neocentrophyidae have middorsal spines restricted to the last segments (10-11 in males, 11 in females), having spinose processes on the remaining ones. Pycnophyidae do not bear spines; instead they present middorsal processes, middorsal elevations or no middorsal specializations at all (See Appendix 1 for detailed information).

- *Lateral terminal spines on the last trunk segment*: Most allomalorhagids bear lateral terminal spines on the last trunk segment, a feature widespread in most kinorhynch species. The species of the genera *Dracoderes*, *Franciscideres*, *Pycnophyes*, *Paracentrophyes* and *Mixtophyes* have a large, conspicuous pair of lateral terminal spines, whereas *Kinorhynchus* and *Neocentrophyes* species were described as lacking such spines (Figs. 18, 19) (Zelinka, 1928; Higgins, 1969, 1983; Sheremetevskij, 1974; Sánchez et al., 2014a in Chapter II).

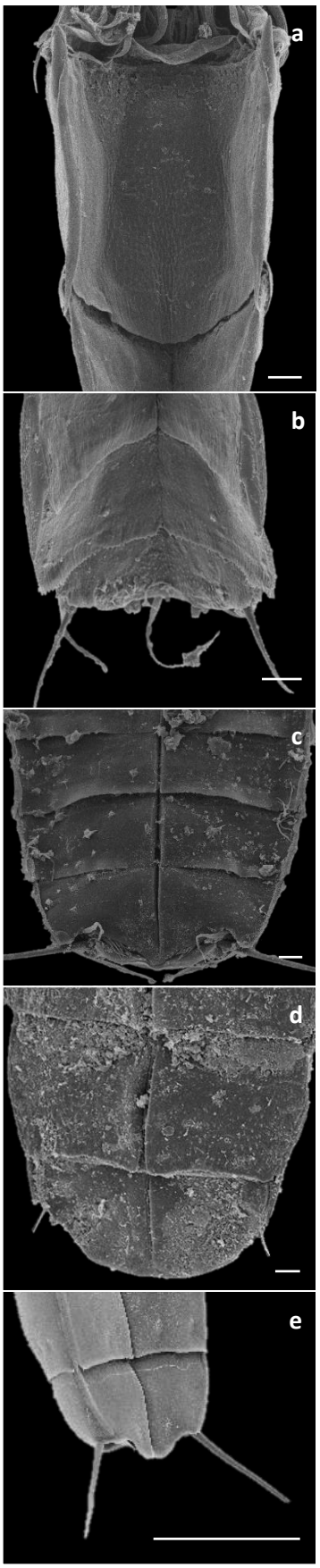


Figure 19. SEM photographs on morphology of Allomalorhagida. a, *Paracentrophyes anurus*, sternal plate organization of segment 1 as partially undivided (scale bar: 10 µm); b, *Paracentrophyes anurus*, sternal plate organization of segment 11 as undivided, single plate (scale bar: 10 µm); c, *Pycnophyes* cf. *ponticus*, sternal plate organization of segment 11 as divided into two plates (scale bar: 10 µm); d, *Kinorhynchus yushini* showing the absence of lateral terminal spines emerging from the last trunk segment (scale bar: 10 µm); e, *Pycnophyes norenburgi*, note the presence of lateral terminal spines emerging from the last trunk segment (scale bar: 100 µm).

Within the Allomalorhagida, the internal relationships of the family Pycnophyidae are still unresolved. The monophyly of the family Pycnophyidae was well supported in all the phylogenetic analyses but all the studies failed to recover its two genera, *Pycnophyes* and *Kinorhynchus*, as monophyletic groupings, but appear as paraphyletic and polyphyletic respectively (Dal Zotto et al., 2013; Yamasaki et al., 2013; Sørensen et al., in press). Both genera have basically the same external morphology, differing just in a single conspicuous trait, the lateral terminal spines, present in *Pycnophyes* and absent in *Kinorhynchus* (Zelinka, 1928; Sheremetevskij, 1974). The paraphyly of *Pycnophyes* was previously suggested by several authors (Higgins, 1962; Brown, 1985; Neuhaus, 1993; Lemburg, 2002) but it could not be tested because of the few amount of available data for the family.

Pycnophyidae is the second largest kinorhynch family, accommodating around one-third of the all known species (Sørensen, 2013; Neuhaus, 2013). Most taxonomic studies on the family were made by Zelinka (1928), Higgins (1983,) and Adrianov (Adrianov and Malakhov, 1999a). It is noteworthy that, except for Zelinka, the number of cyclorhagid species described by these authors is remarkably higher. This apparent lack of interest for pycnophyid species is likely to be originated by the problems that usually arise during the study of the specimens. Most of the morphological characters used traditionally, even in the last available identification key (Adrianov and Malakhov, 1999a), are clearly subjective, ambiguous or only discernible with one particular technique (SEM, DIC). Therefore, the selection and definition of objective morphological characters is strongly needed in order to overcome this problem and encourage the taxonomic researches into this family.

1.8. OBJECTIVES

1. **To standardize an accurate terminology for the specific morphological characters of Allomalorhagida and their position along the trunk:** Many specific cuticular morphological characters of the Allomalorhagida need to be reevaluated and redefined; even more, new objective morphological features should be selected. Subsequently, the positions of these cuticular structures should be able to be plotted unequivocally on a map of a transverse section of an Allomalorhagida specimen, fitting with its generalized triangular trunk shape. This way, a sound basis for future taxonomic work would be stablished.

2. **To Identify the Allomalorhagida species collected in the sampled areas to the lowest taxonomic level:** The new findings of kinorhynch specimens of known species give rise to new citations and biogeographical records. The covered sampling areas are the coast of the Iberian and Korean Peninsula, the Guinea Basin deep-sea, Italy (Naples), East coast of USA (Maine, Massachusetts, Florida), Panama (Bocas del Toro, Naos), Norway (Spegrend), Singapore and Greenland.

3. **To describe new Allomalorhagida taxa from the sampled areas:** New species and/or genera found during the study need accurate description following the taxonomical and nomenclatorial standards stablished in the literature. Such descriptions would increase the knowledge of the diversity and distribution of the phylum worldwide.

4. **To provide a detailed geographic distribution of Allomalorhagida from the Iberian Peninsula:** All the information gathered from all surveys along the coast of the Iberian Peninsula needs to be compiled. Additional rough data on the abundance, types of sediment and depth are included. Moreover, we want to compare the diversity of allomalorhagid species in Atlantic and Mediterranean waters.
5. **To suggest new phylogenetic hypotheses for the Allomalorhagida:** The description of new taxa and the careful revision of type material may reveal new or striking features or even new body arrangements, as for instance the segment plate organization. These observations may give rise to new phylogenetic hypotheses for the whole group at different taxonomical levels.

6. **To test the monophyly of the genera *Pycnophyes* and *Kinorhynchus*:** Within the Allomalorhagida, the monophyly of the family Pycnophyidae is currently well supported

through phylogenetic analyses but its internal relationships are still unresolved, with *Pycnophyes* and *Kinorhynchus* appearing as paraphyletic and polyphyletic respectively. Therefore, addressing their monophyly through molecular and combined molecular and morphological data is hardly needed to follow a classification of the family based on natural groups.

7. **To shed light into the internal relationships of Pycnophyidae for the first time:** Pycnophyidae accommodates around one-third of the all known species, so it is the second largest kinorhynch family. One of the aims of the present Thesis is to further address the internal relationships of the Pycnophyidae gathering for the first time information from all described species. Both morphological and molecular data would be needed in order to integrate a phylogenetic analysis by total evidence. Subsequently, the previously proposed phylogenetic hypotheses would be tested and the classification of the family properly inferred from the phylogenetic analysis to avoid well known systematic errors of the past.

8. **To stablish the evolutionary pathway of morphological characters with phylogenetic relevance:** We need to select the characters bearing relevant phylogenetic information and trace them in the phylogenetic tree in order to test their evolutionary transformation. This way, we can identify and select the apomorphies that may sustain the whole family and the possible recovered ingroups.

2

MATERIAL AND METHODS

2.1. MATERIAL USED IN THE STUDY

Kinorhynch material for the present Thesis was obtained from different sources, both by loan of type material and through numerous collecting campaigns carried out by the meiofauna research group of the UCM and collaborators during the period 1990-2014. Samples were taken along the North, West, South and East of the Spanish coast, as well as from Portugal, France (Banyuls sur Mer), the Guinea Basin deep-sea, South coast of Korea, Italy (Naples), Japan, East coast of USA (Maine, Massachusetts, Florida), Panama (Bocas del Toro, Naos), Norway (Spegrend), Singapore and Greenland. Samples along the North coast of Spain were taken by the RV Cornide de Saavedra during the fisheries campaign CARIOCA90, conducted by the Spanish Institute of Oceanography (GªOrdóñez et al., 2008). Samples from Guinea Basin deep-sea were collected during the R/V Meteor DIVA2 M63/2 Cruise.

Around 1350 specimens accommodated in 43 species were sorted from the samples and studied during the preparation of the present Thesis. In addition, all the available type material of species of Pycnophyidae and Neocentrophyidae were checked, a total of 40 type series with hundreds of specimens. Detailed information on the species by area are specified in Table 2. Likewise, information on the samples, localities, extraction, fixation and mounting as well as the type material studied are specified in the Table 3 and in the “Material and Methods” section of each of the papers included as *Results* of the present Thesis.

Table 2. Diversity of species and their distribution of Allomalorhagid kinorhynchs studied in this Thesis, including the material examined in the Appendix X. Only the specimens observed under LM are taken into account.

Species	Area	Number of specimens
Mixtophyes abyssalis	**Guinea Basin** (deep-sea)	3
Paracentrophyes anurus	**Japan:** Amami Island	2
Paracentrophyes quadridentatus	**Spain:** Bilbao, Cantabria, Asturias **Italy:** Naples **Norway:** Spegrend	17
Paracentrophyes predictus	**Panama:** Bocas del toro	13
Pycnophyes almansae	**Spain:** North Galicia, Soth Galicia, Cádiz, Algeciras, Ceuta, Málaga, Granada	14
Pycnophyes aulacodes	**Spain:** Asturias, North Galicia, South Galicia, Málaga, Granada, Girona **Norway:** Spegrend	83
Pycnophyes chalgap	**South Korea**	5
Pycnophyes carinatus	**Spain:** Málaga, Granada, Girona, **France:** Banyuls	6
Pycnophyes communis	**Spain:** Huelva, Cádiz, Almeria, Valencia, Girona. **France:** Banyuls **Portugal:** Faro, Albufeira	81
Pycnophyes cristatus	**South Korea**	3
Pycnophyes dentatus	**Spain:** Asturias, North Galicia, Soth Galicia, Huelva, Cádiz, Algeciras, Ceuta, Málaga, Granada, Almeria, Valencia, Girona **France:** Banyuls **Portugal:** Faro, Albufeira	485
Pycnophyes dolichurus	**Spain:** North Galicia	36
Pycnophyes farinellii	**Guinea Basin** (deep-sea)	2
Pycnophyes flaveolatus	**Spain:** North Galicia, Málaga, Algeciras	53
Pycnophyes frequens	**USA, East coast:** Masachusstes (Woods Hole)	9
Pycnophyes lageria	**Spain:** Cádiz	10
Pycnophyes norenburgi	**USA**, **East coast:** Florida	17
Pycnophyes nubilis	**Guinea Basin** (deep-sea)	1
Pycnophyes pardosi	**South Korea**	10
Pycnophyes cf. *ponticus*	**Spain:** Algeciras, Almeria, Girona **France:** Banyuls **Italy:** Naples	62

Pycnophyes robustus	**Spain:** Algeciras, Málaga, Girona **Italy:** Naples **Portugal:** Faro	88
Pycnophyes rugosus	**Italy:** Naples	6
Pycnophyes smaug	**South Korea**	7
Pycnophyes tubuliferus	**Japan:** Okinawa **South Korea**	8
Pycnophyes zelinkaei	**Spain:** Algeciras, Ceuta, Málaga, Girona **Italy:** Naples **Norway:** Spegrend	27
Kinorhynchus mainensis	**USA**, **East coast:** Masachusstes (Woods Hole)	5
Pycnophyes greenlandicus	**Greenland:** Independence Fjord, New Polinia	5
Pycnophyes cf. *arctous*	**Greenland:** Independence Fjord , New Polinia	6
Pycnophyes oshoroensisis	**Japan:** Oshoro	1
Pycnophyes sp. 2012a (sp 1)	**Japan:** Okinawa	3
Pycnophyes sp. 2012b (sp2)	**Japan:** Hokkaido	3
Kynorphynchus yushini	**Japan:** Oshoro	4
Pycnophyes sp6	**Greenland:** New Polinia	19
Pycnophyes sp7	**Norway:** Spegrend	3
Pycnophyes sp8	**Norway:** Spegrend	9
Pycnophyes sp9	**Panama:** Bocas del Toro	4
Pycnophyes sp10	**Panama:** Bocas del Toro	2
Pycnophyes sp11	**Singapore**	3
Kinorynchus sp2	**Panama:** Bocas del Toro	6
Kinorynchus sp3	**Panama:** Bocas del Toro	2
Kinorynchus sp4	**Panama:** Naos	6
Kinorynchus sp5	**Panama:** Naos	2

Table 3. Material examined for the present Thesis, including vouchers, additional material collected in sampling campaings and loaned type material from scientific collections (Natural History Museum of Copenhagen and Smithsonian Institution). Details of the microscopic techniques and main references used for each species are included. Abbreviations: *Material collected from the type locality; LM, light microscopy examinations on fixed material; lost, type material does not exist; n.a.loan, type material no available for loan; SEM, scanning electron microscopy examinations.

Species	Type	Voucher	Additional	LM	SEM	Literature
E. sensibilis Adrianov et al., 2002	-	X	-	X	-	-
E. rex Lundbye et al., 2011	-	X	-	X	-	-
Pa. anurus Sørensen et al., 2010	-	X	-	X	-	-
Pa. quadridentatus Zelinka, 1928	lost	X	-	X*	-	-
Pa. praedictus Higgins, 1983	X	-	X	X	X	Higgins, 1983
M. abyssalis Sánchez et al., 2014	X	-	-	-	-	Sánchez et al., 2014a
N. intermedius Higgins, 1969	X	-	-	-	-	Higgins, 1969
N. satyai Higgins, 1969	X	-	-	-	-	Higgins, 1969
P. rugosus Zelinka, 1928	lost	X	-	X*	-	-
P. ponticus Zelinka, 1928	lost	X	-	X*	-	-
P. flaveolatus Zelinka, 1928	lost	X	-	X*	-	-
P. oshoroensisis Yamasaki et al., 2012	-	X	-	X*	-	-
P. sp. 2012a (sp1)	-	X	-	X	-	-
P. sp. 2012b (sp2)	-	X	-	X	-	-
P. dentatus Reinhard, 1881	lost	X	-	X	-	-
P. robustus Zelinka, 1928	lost	X	-	X*	-	-
P. zelinkaei Southern, 1914	lost	X	-	X	-	-
P. tubuliferus Adrianov, 1989	-	X	-	X	-	-
P. communis Zelinka, 1908	lost	-	Italy, Spain	X*	X	Zelinka, 1928
P. greenlandicus Higgins and Kristensen, 1988	X	-	-	-	-	Higgins and Kristensen, 1988
P. kielensis Zelinka, 1928	lost	-	Germany	-	X*	Zelinka, 1928; Neuhaus, 1993
K. giganteus Zelinka, 1928	lost	-	Italy	-	X*	Zelinka, 1928
K. yushini Adrianov, 1989	-	X	-	X	-	-
P. almansae Sánchez et al., 2014	X	-	Spain	X*	X*	Sánchez et al., 2014c
P. carinatus Zelinka, 1928	lost	-	Spain	X	X	Zelinka, 1928
P. chalgap Sánchez et al., 2013	X	-	South Korea	-	X*	Sánchez et al., 2013
P. cristatus Sánchez et al., 2013	X	-	South Korea	-	X	Sánchez et al., 2013
P. dolichurus Sánchez et al., 2011	X	-	Spain	X*	X*	Sánchez et al., 2011
P. farinellii Sánchez et al., 2014	X	-	-	-	-	Sánchez et al., 2014b
P. frequens Blake, 1930	X	-	USA	X	X	Blake, 1930; Herranz et al., 2014
P. lageria Sánchez et al., 2014	X	-	South Korea	X*	X*	Sánchez et al., 2014c
P. norenburgi Herranz et al., 2014	X	-	USA	X*	X*	Herranz et al., 2014
P. nubilis Sánchez et al., 2014	X	-	-	-	-	Sánchez et al., 2014b
P. smaug Sánchez et al., 2013	X	-	South Korea	-	X*	Sánchez et al., 2013
P. sp. nov. 4	-	-	Japan	X	-	-
P. argentinensis Martorelli and Higgins, 2004	X	-	-	-	-	Martorelli and Higgins, 2004
P. beaufortensis Higgins, 1964	X	-	-	-	-	Higgins, 1964
P. ecphantor Higgins, 1983	X	-	-	-	-	Higgins, 1983
P. borealis Higgins and Korczynski, 1990	X	-	-	-	-	Higgins and Korczinski, 1990
P. corrugatus Higgins, 1983	X	-	-	-	-	Higgins, 1983
P. cryopygus Higgins and Kristensen, 1988	X	-	-	-	-	Higgins and Kristensen, 1988
P. egyptensis Higgins, 1966	X	-	-	-	-	Higgins, 1966
P. emarginatus Higgins, 1983	X	-	-	-	-	Higgins, 1983
P. iniorhaptus Higgins, 1983	X	-	-	-	-	Higgins, 1983
P. longicornis Higgins, 1983	X	-	-	-	-	Higgins, 1983
P. neuhausi Martorelli and Higgins, 2004	X	-	-	-	-	Martorelli and Higgins, 2004
P. australensis Lemburg, 2002	X	-	-	-	-	Lemburg, 2003
P. chukchiensis Higgins, 1991	X	-	-	-	-	Higgins, 1991
P. abyssorum Adrianov and Maiorova, 2015	n.a.loan	-	-	-	-	Adrianov and Maiorova, 2015
P. aulacodes Sánchez et al., 2011	X	-	Spain	X*	X*	Sánchez et al., 2011
P. pardosi Sánchez et al., 2013	X	-	South Korea	-	X*	Sánchez et al., 2013

P. sp. nov. 5	-	-	Japan	X	-	-
K. apotomus Higgins, 1983	X	-	-	-	-	Higgins, 1983
K. belizensi Higgins, 1983	X	-	-	-	-	Higgins, 1983
K. deirophorus Higgins, 1983	X	-	-	-	-	Higgins, 1983
K. distentus Higgins, 1983	X	-	-	-	-	Higgins, 1983
K. erismatus Higgins, 1983	X	-	-	-	-	Higgins, 1983
K. fimbriatus Higgins, 1982	X	-	-	-	-	Higgins, 1982
K. langi Higgins, 1964	X	-	-	-	-	Higgins, 1982
K. mainensis Blake, 1930	X	-	-	-	X*	Blake, 1930
K. phyllotropis Brown and Higgins, 1983	X	-	-	-	-	Brown and Higgins, 1983
K. stenopygus Higgins, 1983	X	-	-	-	-	Higgins, 1983
K. trisetosus Higgins, 1983	X	-	-	-	-	Higgins, 1983
P. arctous Adrianov, 1999	n.a.loan	-	-	-	-	Adrianov and Malakhov, 1999
P. barentsi Adrianov, 1999	n.a.loan	-	-	-	-	Adrianov and Malakhov, 1999
P. calmani Southern, 1914	lost	-	-	-	-	Southern, 1914; Zelinka, 1928
P. canadensis Higgins and Korczynski, 1990	n.a.loan	-	-	-	-	Higgins and Korczinski, 1990
P. chilensis Lang, 1953	n.a.loan	-	-	-	-	Lang, 1953
P. faveolus Brown, 1985	n.a.loan	-	-	-	-	Brown, 1985
P. furugelmi Adrianov, 1999	n.a.loan	-	-	-	-	Adrianov and Malakhov, 1999
P. galtsovae Adrianov, 1999	n.a.loan	-	-	-	-	Adrianov and Malakhov, 1999
P. maximus Reimer, 1963	lost	-	-	-	-	Reimer, 1963
P. mokievskii Adrianov, 1995	n.a.loan	-	-	-	-	Adrianov, 1995
P. newguiniensis Adrianov, 1999	n.a.loan	-	-	-	-	Adrianov and Malakhov, 1999
P. newzealandiensis Adrianov, 1999	n.a.loan	-	-	-	-	Adrianov and Malakhov, 1999
P. odhneri Lang, 1949	n.a.loan	-	-	-	-	Lang, 1949
P. parasanjuanensis Adrianov and Higgins, 1996	n.a.loan	-	-	-	-	Adrianov and Higgins, 1996
P. sanjuanensis Higgins, 1961	n.a.loan	-	-	-	-	Higgins, 1961
P. schornikovi Adrianov, 1999	n.a.loan	-	-	-	-	Adrianov and Malakhov, 1999
P. sculptus Lang, 1949	n.a.loan	-	-	-	-	Lang, 1949
P. spitsSpegrendsis Adrianov, 1995	n.a.loan	-	-	-	-	Adrianov, 1995
K. anomalus Lang, 1953	n.a.loan	-	-	-	-	Lang, 1953
K. cataphractus Higgins, 1961	n.a.loan	-	-	-	-	Higgins, 1961; Adrianov and Malakhov, 1999
K. ilyocryptus Higgins, 1961	n.a.loan	-	-	-	-	Higgins, 1961; Boykin, 1965; Adrianov and Malakhov, 1999
K. paraneapolitanus Sheremetevsky, 1974	n.a.loan	-	-	-	-	Sheremetevsky, 1974; Higgins and Adrianov, 1999
K. spinosus Lang, 1949	n.a.loan	-	-	-	-	Lang, 1949
K. rabaulensis Adrianov, 1999	n.a.loan	-	-	-	-	Adrianov and Malakhov, 1999
Pycnophyes sp3	-	-	X	X	X	-
Pycnophyes sp6	-	-	X	X	X	-
Pycnophyes sp7	-	-	X	X	X	-
Pycnophyes sp8	-	-	X	X	X	-
Pycnophyes sp9	-	-	X	X	X	-
Pycnophyes sp10	-	-	X	-	X	-
Pycnophyes sp11	-	-	X	X	X	-
Kinorynchus sp2	-	-	X	X	X	-
Kinorynchus sp3	-	-	X	X	X	-
Kinorynchus sp4	-	-	X	X	X	-
Kinorynchus sp5	-	-	X	X	X	-

2.2. SAMPLING, EXTRACTION AND FIXATION

Kinorhynchs occur on the upper centimeters of the sediment. The “Higgins Meiobentic Dredge” has revealed as the best tool to collect such a surface layer (Higgins, 1964; Higgins and Thiel, 1988; Sørensen and Pardos, 2008). It is built as a sledge-like device with a pair of parallel cutting-

blades between two runners attached to a canvas-protected 62 µm plankton net. Although this kind of dredge has been used in most of the sampling campaigns, the marine sediment may be collected through different ways and procedures. Samples from the RV Cornide de Saavedra were collected using a cylindrical collecting tool (15 cm diameter and 40 cm length) attached to a fisheries bottom trawl (GªOrdóñez et al., 2008); samples from Guinea Basin deep-sea were collected using a multicorer device during the R/V Meteor DIVA2 M63/2 Cruise; samples from Norway were collected through a Van Veen dredge; and samples from Korea were taken with a Smith-MacIntyre Grab or a box corer.

Subsequently the sediment was processed following the "Bubbling and Blot" method as developed and modified by Higgins (1988) (Higgins, 1964; Higgins and Thiel, 1988; Sørensen and Pardos, 2008). The sediment should be firstly diluted with seawater into a bucket and stirred to reach a homogeneous texture. Then, the whole content of the bucket is poured out strongly and repeatedly into another bucket creating as much splashing and turbulence as possible. The mixture should stand until a thin layer of water appears in the surface by decantation of the sediment. The efficiency of this method is based on the hydrophobic condition of the Kinorhynch cuticle: the animals stick to the bubbles generated by the turbulences and are carried out to the surface, where they stay trapped by the surface tension. Consequently, the specimens are picked up through a piece of copying paper gently placed on the surface, where they stay attached. Lastly, the animals are transferred from the paper to a plankton net of 62 µm, the familiarly so-called "mermaid bra", using a spray of seawater. The specimens so recovered were either fixed directly and stored or sorted alive from the remaining hard meiofauna under a stereomicroscope. An Irwin loop was used for sorting the specimens, which consists on a twisted nickel-cadmium thread ending in a minute loop where the animals stay trapped by the surface tension. Kinorhynchs for morphological researches were usually fixed in 4% formalin and stored in ethylene glycol, whereas those specimens used for molecular work were preserved in 100% ethanol.

2.3. PROCEDURES FOR MICROSCOPY

Identification of specimens to the lowest taxonomic level reveals as indispensable to accomplish any kind of study. The minute size of kinorhynchs makes the use of combined microscopical techniques the basic tool for taxonomy, biogeography, morphology and so on.

Specimens for morphological studies using light microscopy (LM) were dehydrated through a graded ethanol series and transferred to 100% glycerin. Around 1200 specimens were mounted preferably in dorsoventral position, either on regular slides or on Cobb slides. These latter consist on

an aluminum frame that holds the specimen between two coverslips, allowing observation of both sides of the specimen (Higgins and Thiel, 1988). Initially, the mounting media was a version of Hoyer's medium (Higgins and Thiel, 1988) modified to avoid excess of transparency through time. However, long term conservation of Hoyer mounted specimens revealed still problematic by crystallization and crackling. Consequently, the chosen medium was Fluoromount G®, with high clarity properties for observation and good expectatives of conservation with no damage for the specimens.

Whole mounted specimens were examined using two different Olympus® BX51 light microscopes with differential interference contrast optics (DIC). The microscopes were equipped with Olympus DP20 and DP70 cameras and the DP Controller and Cell^A® and Cell^D® software for measurements and photographs. Line art figures were made with Adobe Illustrator® CS4 and CS6.

Additional specimens (around 150) were selected for scanning electron microscopy (SEM). Firstly, the specimens were dehydrated through a graded ethanol series, and then transferred to 100% acetone and critical point dried. The dried specimens were then mounted on aluminum stubs, sputter coated with platinum or gold and examined and photographed with either a JEOL JSM-6400 or JEOL JSM-6335F field emission scanning electron microscopes at the Centro Nacional de Microscopía Electrónica (UCM) and the Natural History Museum of Copenhagen.

Terminology related to the distribution of cuticular features in allomalorhagids follows the standardization criteria established by Sánchez et al. (2011) (see Chapter I). Terminology shared by both allomalorhagid and cyclorhagid kinorhynchs follows Sørensen and Pardos (2008). The number and distribution of introvert appendages has been mapped using polar diagrams standardized by Higgins (1990) and updated in Sørensen and Pardos (2008). The description of scalids follows the standards established by Brown (1989) and Neuhaus (2013).

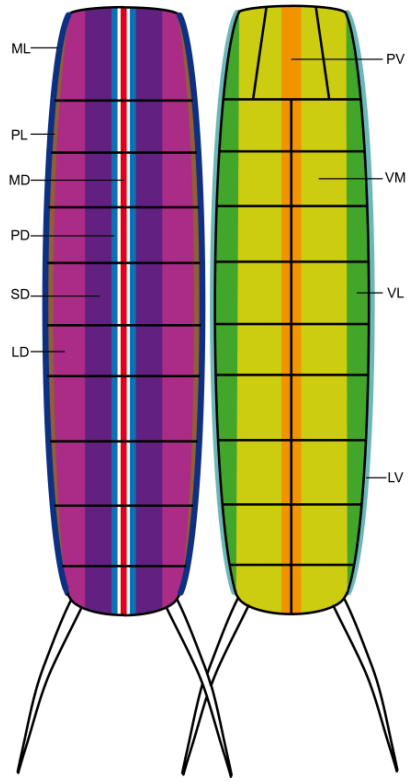


Figure 20. Schematic longitudinal section along the trunk of a common allomalorhagid kinorhynch. Drawings display positions of the cuticular characters in dorsal and ventral view. Abbreviations: LD, laterodorsal; LV, lateroventral; MD, middorsal; ML, midlateral; PD, paradorsal; PL, paralateral; PV, paraventral; SD, subdorsal; VL, ventrolateral; VM, ventromedial.

Even though the systematics within Pycnophyidae has been reorganized very recently (see Chapter III), most of the publications compiled in this Thesis were performed earlier, following the old classification valid at that time. Such classification divided the family into two genera, *Pycnophyes* and *Kinorhynchus*. With the aim of avoid confusion between the species names that appear in the publications and the newly stablished ones, we decided to follow the old classification throughout the present Thesis. The only obvious exception is the publication included in Chapter III, in which the new classification is enclosed. Nevertheless, the list of all species of Pycnophyidae including the old and the new names is also included in Table 4 to make reading easier.

Table 4. List of species of Pycnophyidae, including the old and the new name for each species.

Before this study	This study
K. anomalus Lang, 1953	*Cristaphyes anomalus* n. comb. (Lang, 1953)
K. apotomus Higgins, 1983	*Godzilliphyes apotomus* n. comb. (Higgins, 1983)
K. belizensis Higgins, 1983	*Cristaphyes belizensis* n. comb. (Higgins, 1983)
K. cataphractus Higgins, 1961	*Higginsia cataphracta* n. comb. (Higgins, 1961)
K. deirophorus Higgins, 1983	*Fujuriphyes deirophorus* n. comb. (Higgins, 1983)
K. distentus Higgins, 1983	*Fujuriphyes distentus* n. comb. (Higgins, 1983)
K. erismatus Higgins, 1983	*Higginsia erismata* n. comb. (Higgins, 1983)
K. fimbriatus Higgins, 1982	*Planolimbus fimbriatus* n. comb. (Higgins, 1982)
K. giganteus Zelinka, 1928	*Pycnophyes giganteus* n. comb. (Zelinka, 1908)
K. ilyocryptus Higgins, 1961	*Pycnophyes ilyocryptus* Higgins, 1961
K. langi Higgins, 1964	*Planolimbus langi* n. comb. (Higgins, 1964)
K. mainensis Blake, 1930	*Planolimbus mainensis* n. comb. (Blake, 1930)
K. paraneapolitanus Sheremetevskij, 1974	*Pycnophyes paraneapolitanus* n. comb. (Sheremetevskij, 1974)
K. phyllotropis Brown and Higgins, 1983	*Cristaphyes phyllotropis* n. comb. (Brown and Higgins, 1983)
K. rabaulensis Adrianov 1999	*Cristaphyes rabaulensis* n. comb. (Adrianov, 1999 in Adrianov and Malakhov, 1999)
K. spinosus Lang, 1949	*Cristaphyes spinosus* n. comb. (Lang, 1949)
K. stenopygus Higgins, 1983	*Pycnophyes stenopygus* Higgins, 1983
K. trisetosus Higgins, 1983	*Higginsia trisetosa* n. comb. (Higgins, 1983)
K. yushini Adrianov, 1989	*Cristaphyes yushini* n. comb. (Adrianov, 1989)
P. sp. 2012a	*Pycnophyes* 2012a
P. sp. 2012b	*Krakenella* 2012b
P. abyssorum Adrianov and Maiorova, 2015	*Cristaphyes abyssorum* n. comb. (Adrianov and Maiorova, 2015)
P. almansae Sánchez et al., 2014	*Godzilliphyes almansae* n. comb. (Sánchez et al., 2014)
P. arctous Adrianov, 1999	*Cristaphyes arctous* n. comb. (Adrianov, 1991 in Adrianov and Malakhov, 1991)
P. argentinensis Martorelli and Higgins, 2004	*Gymnophyes argentinensis* n. comb. (Martorelli and Higgins, 2004)
P. aulacodes Sánchez et al., 2011	*Pycnophyes aulacodes* Sánchez et al., 2011
P. australensis Lemburg, 2002	*Setaphyes australensis* n. comb. (Lemburg, 2002)
P. barentsi Adrianov, 1999	*Gymnophyes barentsi* n. comb. (Adrianov, 1999 in Adrianov and Malakhov, 1999)
P. beaufortensis Higgins, 1964	*Pycnophyes beaufortensis* Higgins, 1964
P. borealis Higgins and Korczynski, 1989	*Gymnophyes borealis* n. comb. (Higgins and Korczynski, 1989)
P. calmani Southern, 1914	*Pycnophyes calmani* Southern, 1914
P. canadensis Higgins and Korczynski, 1989	*Gymnophyes canadensis* n. comb. (Higgins and Korczynski, 1989)
P.carinatus Zelinka, 1928	*Cristaphyes carinatus* n. comb. (Zelinka, 1928)
P. chalgap Sánchez et al., 2013	*Planolimbus chalgap* n. comb. (Sánchez et al., 2013)
P. chilensis Lang, 1953	*Cristaphyes chilensis* n. comb. (Lang, 1953)
P. chukchiensis Higgins, 1991	*Cristaphyes chukchiensis* n. comb. (Higgins, 1991)
P. communis Zelinka, 1908	*Pycnophyes communis* Zelinka, 1908
P. corrugatus Higgins, 1983	*Planolimbus corrugatus* n. comb. (Higgins, 1983)
P. cristatus Sánchez et al., 2013	*Cristaphyes cristatus* n. comb. (Sánchez et al., 2013)
P. cryopygus Higgins and Kristensen, 1988	*Cristaphyes cryopygus* n. comb. (Higgins and Kristensen, 1988)
P. dentatus Reinhard, 1881	*Setaphyes dentatus* n. comb. (Reinhard, 1881)
P. dolichurus Sánchez et al., 2011	*Higginsia dolichura* n. comb. (Sánchez et al., 2010)
P. ecphantor Higgins, 1983	*Planolimbus ecphantor* n. comb. (Higgins, 1983)
P. egyptensis Higgins, 1966	*Godzilliphyes egyptensis* n. comb. (Higgins, 1966)

P. emarginatus Higgins, 1983	*Planolimbus emarginatus* n. comb. (Higgins, 1983)
P. farinellii Sánchez et al., 2014	*Gymnophyes farinellii* n. comb. (Sánchez et al., 2014)
P. faveolus Brown, 1985	*Planolimbus faveolus* n. comb. (Brown, 1999 in Adrianov and Malakhov, 1999)
P. flaveolatus Zelinka, 1928	*Setaphyes flaveolatus* n. comb. (Zelinka, 1928)
P. frequens Blake, 1930	*Pycnophyes frequens* Blake, 1930
P. furugelmi Adrianov, 1999	*Cristaphyes furugelmi* n. comb. (Adrianov, 1999 in Adrianov and Malakhov, 1999)
P. galtsovae Adrianov, 1999	*Gymnophyes galtsovae* n. comb. (Adrianov, 1999 in Adrianov and Malakhov, 1999)
P. greenlandicus Higgins and Kristensen, 1988	*Gymnophyes greenlandicus* n. comb. (Higgins and Kristensen, 1988)
P. iniorhaptus Higgins, 1983	*Setaphyes iniorhaptus* n. comb. (Higgins, 1983)
P. kielensis Zelinka, 1928	*Setaphyes kielensis* n. comb. (Zelinka, 1928)
P. lageria Sánchez et al., 2014	*Planolimbus lageria* n. comb. (Sánchez et al., 2014)
P. longicornis Higgins, 1983	*Cristaphyes longicornis* n. comb. (Higgins, 1983)
P. maximus Reimer, 1963	*Gymnophyes maximus* n. comb. (Reimer, 1963)
P. mokievskii Adrianov, 1995	*Gymnophyes mokievskii* n. comb. (Adrianov, 1995)
P. neuhausi Martorelli and Higgins, 2004	*Godzilliphyes neuhausi* n. comb. (Higgins, 2004 in Martorelli and Higgins, 2004)
P. newguiniensis Adrianov, 1999	*Godzilliphyes newguiniensis* n. comb. (Adrianov, 1999 in Adrianov and Malakhov, 1999)
P. newzealandiensis Adrianov, 1999	*Godzilliphyes newzealandiensis* n. comb. (Adrianov, 1999 in Adrianov and Malakhov, 1999)
P. norenburgi Herranz et al., 2014	*Pycnophyes norenburgi* Herranz et al., 2014
P. nubilis Sánchez et al., 2014	*Cristaphyes nubilis* n. comb. (Sánchez et al., 2014)
P. odhneri Lang, 1949	*Cristaphyes odhneri* n. comb. (Lang, 1949)
P. oshoroensis Yamasaki et al., 2012	*Pycnophyes oshoroensis* Yamasaki et al., 2012
P. parasanjuanensis Adrianov and Higgins, 1996	*Krakenella parasanjuanensis* n. comb. (Adrianov and Higgins, 1996)
P. pardosi Sánchez et al., 2013	*Planolimbus pardosi* n. comb. (Sánchez et al., 2013)
P. ponticus Zelinka, 1928	*Fujuriphyes ponticus* n. comb. (Zelinka, 1928)
P. robustus Zelinka, 1928	*Godzilliphyes robustus* n. comb. (Zelinka, 1928)
P. rugosus Zelinka, 1928	*Fujuriphyes rugosus* n. comb. (Zelinka, 1928)
P. sanjuanensis Higgins, 1961	*Krakenella sanjuanensis* n. comb. (Higgins, 1961)
P. schornikovi Adrianov, 1999	*Pycnophyes schornikovi* Adrianov, 1999 in Adrianov and Malakhov, 1999
P. sculptus Lang, 1949	*Planolimbus sculptus* n. comb. (Lang, 1949)
P. smaug Sánchez et al., 2013	*Gymnophyes smaug* n. comb. (Sánchez et al., 2013)
*P.*sp. nov. 4	*Fujuriphyes* sp nov 4
*P.*sp. nov. 5	*Krakenella* sp. nov. 5
P. spitsbergensis Adrianov, 1995	*Gymnophyes spitsbergensis* n. comb. (Adrianov, 1995)
P. tubuliferus Adrianov, 1989	*Pycnophyes tubuliferus* Adrianov, 1989
P. zelinkaei Southern, 1914	*Pycnophyes zelinkaei* Southern, 1914

2.4. METHODS FOR PHYLOGENY

Total genomic DNA was extracted from individual specimens using a DNeasy Tissue Kit (Qiagen, Tokyo), following the protocol of Yamasaki et al. (2013). After DNA extraction, the exoskeleton of each specimen was picked up and used as hologenophore. This procedure allows the use of combined molecular and morphological information from the same voucher specimen (see "Materials and Methods" section in Chapter III). Nuclear 18S rRNA (18S), 28S rRNA (28S) genes, and mitochondrial cytochrome *c* oxidase subunit I gene (COI) were amplified by the same PCR conditions as in Yamasaki and Fujimoto (2014). All nucleotide sequences were determined by direct sequencing with a BigDye Terminator Kit ver. 3.1 (Life Technologies, Co., USA) and a 3730 DNA Analyzer (Life Technologies, Co., USA). The sequence fragments were assembled by MEGA 5 (Tamura et al., 2011). The 18S rRNA and 28S rRNA gene fragments were aligned independently in the software MAFFT ver. 7.058 (Katoh et al., 2002; Katoh et al., 2010). Subsecuently, gaps were treated using the software TrimAl (Capella-Gutiérrez et al., 2009) or Gblocks ver. 0.91b (Castresana, 2000).

Morphological data were registered for a total of 87 species (79 Pycnophyidae and eight outgroups), whereas molecular data were obtained for 19 taxa (15 Pycnophyidae and 4 outgroups). The morphological data were coded after the hologenophore vouchers and all the available type material; otherwise morphological characters were coded either from information on the literature or taken from additional specimens that were identified and studied during the elaboration of the present Thesis.

Morphological data was analyzed under parsimony using the software PAUP ver. 4.0b10 (Swofford, 2003). The combined datasets (molecular+morphology) were analyzed under model-based methods (Maximum likelihood and Bayesian) and dynamic homology and parsimony. Dynamic homology was applied using the software POY ver. 4.1.2. (Wheeler et al., 2006; Varón et al., 2010). Maximum Likelihood analyses were calculated using RAxML ver. 8.1.11 (Stamatakis et al., 2008). Bayesian analyses were computed with MrBayes ver. 3.2.2 (Ronquist and Huelsenbeck, 2003). Except for POY analyses, all phylogenetic analyses were run using the Cipres Phylogenetic Portal (Miller et al., 2010). Ancestral character states were reconstructed with the software Mesquite ver. 3.0.1 (Maddison and Maddison, 2007), using parsimony as optimal criteria.

3

RESULTS

Chapter I

Taxonomy of Pycnophyidae

Pycnophyes dolichurus sp. nov. and *P. aulacodes* sp. nov. (Kinorhyncha, Homalorhagida, Pycnophyidae), two new kinorhynchs from Spain with a reevaluation of homalorhagid taxonomic characters

Nuria Sánchez, Fernando Pardos, María Herranz, Jesús Benito

RESUMEN: ***Pycnophyes dolichurus* sp. nov. and *P. aulacodes* sp. nov. (Kinorhyncha, Homalorhagida, Pycnophyidae), dos nuevos kinorrincos de España con una reevalucíon de los caracteres taxonómicos de los homalorágidos.** – Se describen dos nuevas especies de kinorrincos del género *Pycnophyes* recolectados en el océano Atlántico, Noroeste de España, y del mar Mediterráneo, Este de España, mediante microscopía de interferencia de contraste y microscopía electrónica de barrido (SEM): *Pycnophyes dolichurus* sp. y. and *P. aulacodes* sp. nov. Las estructuras cuticulares utilizadas como caracteres taxonómicos en homalorhagidos son discutidas y reevaluadas. Además, se define la localización longitudinal de las estructuras cuticulares a lo largo del tronco, así como que se estandariza la terminología de las posiciones. Se revisa la distribución del género *Pycnophyes* en las costas europeas, lo que reveló un escaso conocimiento de la biogeografía de los kinorrincos, probablemente debido a muestreos incompletos.

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ORIGINAL ARTICLE

Pycnophyes dolichurus sp. nov. and *P. aulacodes* sp. nov. (Kinorhyncha, Homalorhagida, Pycnophyidae), two new kinorhynchs from Spain with a reevaluation of homalorhagid taxonomic characters

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Abstract Two new species of the kinorhynch genus *Pycnophyes* are described from the Atlantic Ocean, Northwest Spain, and the Mediterranean Sea, East of Spain, using differential interference contrast microscopy and scanning electron microscopy (SEM): *Pycnophyes dolichurus* sp. nov. and *P. aulacodes* sp. nov. Taxonomic characters from cuticular structures in homalorhagids are discussed and reevaluated. The longitudinal positions of cuticular structures along the trunk are furthermore defined, and the positional terminology is standardized. The distribution of the genus *Pycnophyes* in European waters is revised, revealing a poor knowledge of kinorhynch biogeography, probably due to incomplete sampling.

Keywords *Pycnophyes* · Kinorhyncha · Meiofauna · Geographic distribution · Cuticular characters · Taxonomy

Introduction

Kinorhyncha is a phylum of meiobenthic animals. Their total lengths never exceed more than 1 mm, and they are found in marine or estuarine sediments exclusively, from coarse sand or shell gravel to very fine mud (Higgins 1964a, 1983, 1988). Their body is covered by cuticle and divided into an anterior, eversible introvert with scalids, a neck and a trunk with 11 segments. For a description of the general anatomy and taxonomic characters of kinorhynchs, see Higgins (1983), Kristensen and Higgins (1991), and Sørensen and Pardos (2008).

The order Homalorhagida Zelinka, 1896 comprises two families: Neocentrophyidae Higgins, 1969 and Pycnophyidae Zelinka, 1896, of which the latter is characterized by having the first trunk segment consisting of one tergal and one sternal plate. The sternal plate may be either partially or completely divided into three additional subunits: two episternal plates and one midsternal. Segments 2–11 are composed of one tergal and two sternal plates. Unlike many other species of kinorhynchs, members of the family Pycnophyidae have no middorsal spines on the trunk segments; however, many species present keel-like middorsal, posteriorly directed processes. Furthermore, cuticular setae may be present in various positions (Higgins 1983, 1988). The family Pycnophyidae comprises two genera: *Pycnophyes* and *Kinorhynchus*, which can be discriminated by the absence of lateral terminal spines in the latter, whereas species of *Pycnophyes* show lateral terminal spines on segment 11 in both sexes. Males of *Pycnophyes* can be distinguished from females by the presence of ventrolateral tubules on segment 2 and penile spines on the terminal trunk segment (Zelinka 1928).

Currently, the genus *Pycnophyes* includes 43 species. Eleven species have been recorded from European waters: 7 in the Atlantic (Table 1) and 8 in the Mediterranean (Table 2), with 4 species being present in both waters. Furthermore, species of other homalorhagid genera have been found in European waters: *Kinorhynchus* and *Paracentrophyes* have been reported from the Atlantic and Mediterranean (see references in Higgins and Adrianov 1991; Adrianov and Malakhov 1999; Sørensen and Pardos 2008; Sørensen et al. 2010). *Pycnophyes quadridentatus* Zelinka, 1928 and *P. flagellatus* Zelinka, 1928 were

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Table 1 Atlantic European species of *Pycnophyes*

Species	Locality	Reference
Pycnophyes zelinkaei Southern, 1914	Ireland, Clew Bay	Southern (1914)
	UK, Fladden	McIntyre (1962)
P. calmani (Southern, 1914)	Ireland, Clew Bay	Southern (1914)
	UK, St. Andrews Bay	Zelinka (1928)
	Ireland, Irish coast	Zelinka (1928)
	Denmark, Gilleleje Flak (Øresund)	Lang (1936)
	Clay Deep, North Sea	Huys and Coomans (1989)
P. dentatus (Reinhard, 1881)	Ireland, Clew Bay	Southern (1914)
	Germany, Kiel Bay	Zelinka (1928)
	Netherlands, Scheveningen	Zaneveld (1938)
	UK, Isle of Man	Bruce et al. (1963)
	Germany, Helgoland, Sylt (North Sea)	Neuhaus (1993)
P. communis Zelinka, 1908	Sweden, Gullmar Fjord	Nyholm (1947)
P. flaveolatus Zelinka, 1928	Sweden, Gullmar Fjord	Nyholm (1947)
	Denmark, Gilleleje Flak (Øresund)	Lang (1936)
P. kielensis Zelinka, 1928	Germany, Kiel Bay	Zelinka (1928)
	Denmark, Vedbæk (Øresund)	Lang (1936)
	Germany, Greifswalder Boden	Reimer (1963)
	Germany, Helgoland, Sylt (North Sea)	Neuhaus (1988)
P. maximus Reimer, 1963	Germany, Kadetrinne	Reimer (1963)

Table 2 Mediterranean European species of *Pycnophyes*

Species	Locality	Reference
Pycnophyes communis Zelinka, 1908	Italy, Naples Gulf	Zelinka (1928)
	Italy, Trieste Gulf	Zelinka (1928)
P. robustus Zelinka, 1928	Italy, Naples Gulf	Zelinka (1928)
	Italy, Trieste Gulf	Zelinka (1928)
P. carinatus Zelinka, 1928	Italy, Naples Gulf	Zelinka (1928)
	Italy, Trieste Gulf	Zelinka (1928)
P. flaveolatus Zelinka, 1928	Italy, Naples Gulf	Zelinka (1928)
	Italy, Trieste Gulf	Zelinka (1928)
P. rugosus Zelinka, 1928	Italy, Naples Gulf	Zelinka (1928)
P. ponticus (Reinhard, 1881)	Ukrania, Odessa, Black Sea	Reinhard (1881)
	Italy, Naples Gulf	Zelinka (1928)
	Rumania, Black Sea	Bãcescu and Bãcescu (1956)
	Bulgaria, Black Sea	Marinov (1964)
	Russia, Black Sea	Sheremetevskij (1974)
P. dentatus (Reinhard, 1881)	Ukrania, Odessa, Black Sea	Reinhard (1881)
	Rumania, Black Sea	Bãcescu and Bãcescu (1956)
	Russia, Black Sea	Sheremetevskij (1974)
P. kielensis Zelinka, 1928	Ukrania, Odessa, Black Sea	Reinhard (1881)
	Rumania, Black Sea	Bãcescu (1968)
	Russia, Black Sea	Sheremetevskij (1974)

originally described from the gulf of Naples (Zelinka 1928), but the two species were subsequently considered conspecies non-*Pycnophyes*, and a new genus, *Paracentrophyes*, was erected to accommodate the new species combination (Higgins 1983).

Along the Spanish coasts, species of *Pycnophyes* have not previously been recorded, and studies on kinorhynchs in Spain have been limited to the cyclorhagid genus *Echinoderes*. *E. canariensis* Greeff, 1869 was described from the Canary Islands but was later considered as *species*

inquirendum (Pardos et al. 1998). *E. dujardinii* was reported from the Baleares Islands (Pagenstecher 1875), and in 1998 two new species were described from the Cantabric Sea (NW Spain): *E. hispanicus* Pardos et al. (1998), and *E. cantabricus* Pardos et al. (1998), followed in 2008 by three additional new species: *E. isabelae* G^{a}Ordóñez et al. (2008), *E. parrai* G^{a}Ordóñez et al. (2008), and *E. neospinosus* G^{a}Ordóñez et al. (2008) (see G^{a}Ordóñez et al. 2008).

The aim of the present paper is to describe two new species of the genus *Pycnophyes* from the Spanish coasts—one from the Atlantic coast and one from the Mediterranean. These species are the first pycnophyids reported for the Iberian Peninsula and the first new species of *Pycnophyes* from the Mediterranean Sea since the times of Zelinka's monography in 1928. Furthermore, the present paper offers a complete and up-to-date overview of the *Pycnophyes* species distribution in the European coasts.

Materials and methods

Specimens of *Pycnophyes* were collected at various localities in the Mediterranean and Atlantic coasts. Data on sampling localities, including position, type of the sediment, and depth are summarized in Table 3. Mediterranean sampling localities included Garrucha (Almería, SE Spain) and Denia (Alicante, E Spain), visited in 1997, and Blanes (Gerona, NE Spain) in 1999. Sampling along the Atlantic coasts included stations in the fjords Ría de Ferrol and Ría de Ares (La Coruña, NW Spain) in 2007 and in Ría de La Coruña and Ría de Ares (La Coruña, NW Spain) in 2008 (Fig. 1). All samples were subtidal, and although exact depths are not available for all sampling stations, the deepest locality was in the Ría de Ares, with 45.4 m.

Sediment samples were taken using a Higgins Meiobenthic Dredge (Higgins 1964b, 1988) that collects the upper centimeters of sediment. The meiofauna was extracted from sediment samples using the bubble and blot method (Higgins 1964b, 1988; Sørensen and Pardos 2008). Samples were fixed with 7–10% formalin and dyed with Rose Bengal. Three hundred kinorhynch specimens were sorted under a dissecting microscope and mounted for LM either on regular slides or on Cobb-slides with either Hoyer's medium or Fluoromount-G®, following standard procedures (Sørensen and Pardos 2008). The specimens were observed and photographed using an Olympus BX51

Table 3 Sampling localities for *Pycnophyes dolichurus* sp. nov. and *P. aulacodes* sp. nov., including number of specimens obtained (*n*), geographic coordinates, type of sediment, depth, and type locality (*)

Sample	*n P. dolichurus*	*n P. aulacodes*	Locality	Coordinates	Sediment	Depth (m)
970324.1B	0	1	Garrucha	37° 9′13.97″N 1°47′ 57.12″W	Coarse sand	–
970325.3B	0	1	Garrucha	37°10′ 55.53″N 1°49′ 30.3″W	Mud	–
970327.2B	0	6	Denia	38° 50′ 14″N 0° 9′ 25″E	Fine sand	–
990324.3B	0	1	Blanes	41° 38.596′N 02° 46.255′E	Midfine sand	–
070626.5	0	1	Ría de Ferrol	43° 28.178′N 008° 14.716′W	Mud	–
070627.1*	0	15	Ría de Ares	43° 25.064′N 008° 16.558′W	Mud	–
070627.3*	13	1	Ría de Ares	43° 24.844′N 008° 17.832′W	Coarse sand	–
070627.5	0	2	Ría de Ferrol San Cristobo	43° 27.887′N 008° 18.118′W	Midfine sand Muddy	–
080403.3	0	1	Ría Coruña	43° 22.208′N 008° 21.177′W	Midfine sand	19
080403.4	0	1	Ría Coruña	43° 22.718′N 008° 21.858′W	Fine sand	27
080403.6	0	1	Ría Coruña	43° 21.697′N 008° 22.713′W	Midfine sand Muddy	19.1
080404.3	21	1	Ría de Ares	43° 25.400′N 008° 20.769′W	Fine sand	45.4
080404.5	0	4	Ría de Ares	43° 23.232′N 008° 15.391′W	Fine sand	13

Fig. 1 Mediterranean and Atlantic sampling localities

microscope equipped with differential interference (DIC) optics. Several specimens were dehydrated through a graded series of ethanol, transferred to acetone, critical point dried, and mounted on aluminum stubs for observation and photography with a JSM 6400 JEOL scanning electron microscope.

Segment numbering follows the terminology established by Neuhaus and Higgins (2002) and applied by Sørensen and Pardos (2008). The present study revealed some inconsistencies in the literature regarding the terminology of systematic characters and their precise location along the body. In order to provide a sound basis for the present and future studies, we marked positions along longitudinal lines or bands on the trunk where taxonomically important cuticular characters usually appear in homalorhagids. These additions to the terminology are in agreement with the corresponding terminology established by Pardos et al. (1998) for cyclorhagid kinorhynchs. However, anatomical differences between cyclorhagids and homalorhagids prevent in some instances the application of the same terms and positions for both groups. Furthermore, and because of the expected variability, some positions refer to longitudinal lines, more fixed and stable, and others to bands or strips, where some slight variation among species or individuals may appear. Following the emended terminology, positions in homalorhagid kinorhynchs are defined as follows (see also Fig. 2).

Dorsal series

Middorsal (*MD*) Line. Referres to structures located dorsal on the midpoint of the tergal plate. This is the highest elevated point on the trunk that appears triangular shaped in cross section. The position can only be observed from the dorsal side of a dorsoventrally mounted specimen.

Paradorsal (*PD*) Line. Referres to structures immediately adjacent to the middorsal position of the segment. The position can only be observed from the dorsal side of a dorsoventrally mounted specimen.

Subdorsal (*SD*) Band. Referres to structures located bilaterally on the dorsalmost 50% of the tergal area

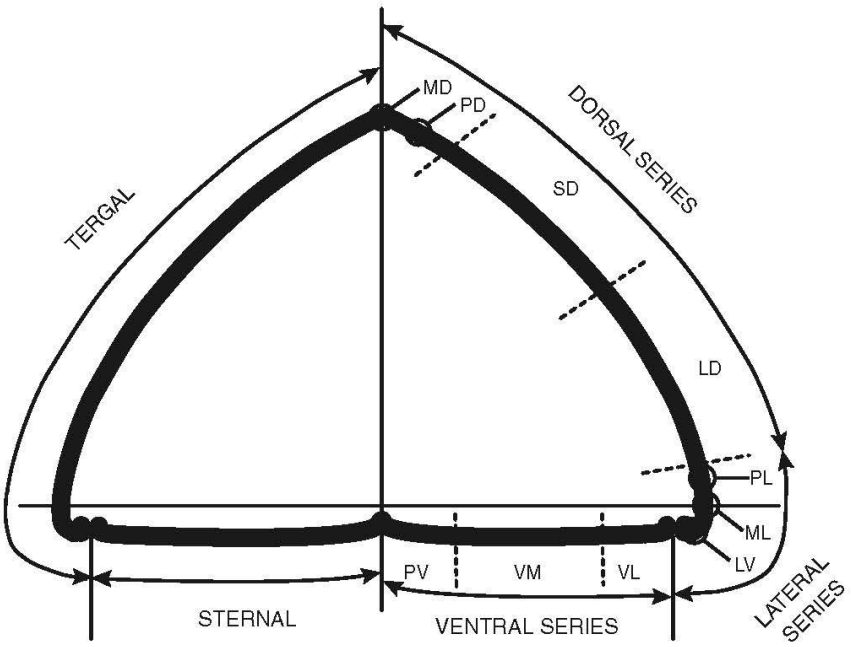

Fig. 2 Schematic transverse section across a trunk segment of a homalorhagid kinorhynch, showing positions of cuticular characters. *Lines* are marked by *circles*. *Bands* are limited by *dotted lines*. *LD* laterodorsal, *LV* lateroventral, *MD* middorsal, *ML* midlateral, *PD* paradorsal, *PL* paralateral, *PV* paraventral, *SD* subdorsal, *VL* ventrolateral, *VM* ventromedial

between the paradorsal position and the widest point of the trunk. The position can only be observed from the dorsal side of a dorsoventrally mounted specimen.

Laterodorsal (*LD*) Band. Referres to structures located bilaterally on the ventralmost 50% of the tergal area between the paradorsal position and the widest point of the trunk. The position can only be observed from the dorsal side of a dorsoventrally mounted specimen.

Positions of dorsal series are the same in both cyclorhagids and homalorhagids.

Lateral series

Paralateral (*PL*) Line. Referres to structures located bilaterally adjacent and dorsal to the midlateral position of the segment. The position can only be observed from the dorsal side of a dorsoventrally mounted specimen.

Midlateral (*ML*) Line. Referres to structures located bilaterally on the tergal plate at the lateral edge of the segment, as seen from both the dorsal and ventral sides. It marks the widest point of the segment.

Lateroventral (*LV*) Line. Referres to structures located bilaterally on the tergal plate, immediately adjacent to the tergosternal junction, as seen from the ventral side of a dorsoventrally mounted specimen.

LV is the ventralmost position of the lateral series in both cyclorhagids and homalorhagids. In homalorhagids, PL is the dorsalmost position of the series, whereas such a position does not apply to the cyclorhagid trunk shape. Hence, ML is the dorsalmost position in the lateral series of cyclorhagids, and they have in addition two positions, lateral accessory (LA) and sublateral (SL), located in between ML and LV. None of these additional positions apply to the more triangular homalorhagid trunk shape. However, cuticular structures have been detected close, but still dorsal, to the ML position (the widest point of the segment). Hence, the new position paralateral (PL) is introduced to comply with structures in this position. In our system, the PL position is related to the ML in the same way as the PD is related to the MD, or the LA is related to LV in cyclorhagids.

Ventral series

Ventrolateral (*VL*) Band. Referres to structures located bilaterally on the sternal plate, adjacent to the tergosternal junction. It extends over the outer longitudinal quarter of the sternal plate. The position can only be observed from the ventral side of a dorsoventrally mounted specimen.

Ventromedial (*VM*) Band. Referres to structures located bilaterally at or near the middle of the sternal plate, between ventrolateral and paraventral bands. The position can only be observed from the ventral side of a dorsoventrally mounted specimen.

Paraventral (*PV*) Band. Referres to structures located bilaterally adjacent to the midventral line of the segment. It extends over the inner quarter of the sternal plate. The position can only be observed from the ventral side of a dorsoventrally mounted specimen.

Positions of ventral series are the same in both homalorhagids and cyclorhagids.

Both the dorsal and lateral series refer to positions on the tergal plate, whereas the ventral series refers to positions on the sternal plates. In the subsequent description, the word 'pair' will refer to bilateral symmetrical structures. When two very closely positioned cuticular structures appear, they will be referred to as 'twins', having their correspondent pair of twin structures on the other side of the animal.

Results

Taxonomic account

Pycnophyes dolichurus sp. nov.

Order Homalorhagida Zelinka, 1896
Suborder Homalorhagae Zelinka, 1896
Family Pycnophyidae Zelinka, 1896
Genus *Pycnophyes* Zelinka, 1907

Examined material

A total of 34 specimens (18 males and 16 females) have been examined with DIC and 5 additional specimens (3 females and 2 males) with SEM. All specimens were collected in Ría de Ares, NW Spain. The type series includes the holotype, an adult female (locality: Ría de Ares, station no. 070627.3, position 43° 24.844′N, 008° 17.832′W, date 2007/06/27), the allotypic adult male (locality: Ría de Ares, station no. 080404.3, position 43° 25.400′N, 008° 20.769′W; date 2008/04/04), and 10 additional paratypes, 5 males and 5 females from the same locality as the allotype. All types of specimens are mounted with Fluoromount-G® and deposited at the Zoological Museum, University of Copenhagen under accession numbers ZMUC KIN-430 (holotype), ZMUC KIN-431 (allotype), and ZMUC KIN-432 to KIN-441 (paratypes). Additional specimens remain in the personal collection of the first author.

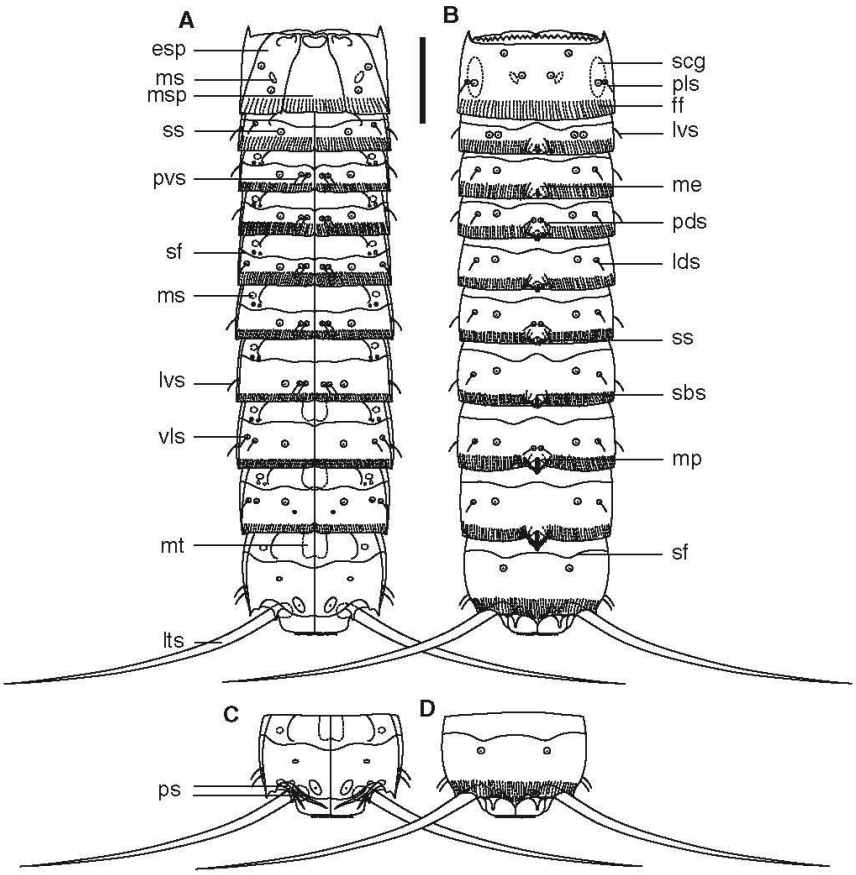

Fig. 3 *Pycnophyes dolichurus* sp. nov. **a** Female, ventral view. **b** Female, dorsal view. **c** Male, ventral view of segments 10 and 11. **d** Male, dorsal view of segments 10 and 11. Scale bar: 100 µm. *esp* episternal plate, *ff* free flap, *lds* laterodorsal seta, *lts* laterоterminal spine, *lvs* lateroventral seta, *me* middorsal elevation, *mp* middorsal process, *ms* muscular scar, *msp* midsternal plate, *mt* anteromesial thickenings of ventral pachycycli (Mittelwülste), *pds* paradorsal seta, *pls* paralateral seta, *ps* penile spine, *pvs* paraventral seta, *sbs* subcuticular structure (atria), *scg* subcuticular gland, *sf* secondary fringe, *ss* sensory spot, *vls* ventrolateral seta

Etymology

The species name, *dolichurus*, is masculine, derived from Greek *dolichos*, long, and *oura*, tail, and refers to the lateral terminal spines—the longest described so far.

Diagnosis

Pycnophyes with middorsal elevations from segments 2–7 that extend into middorsal processes in segments 8 and 9. Anterior margin of tergal plate of first trunk segment strongly denticulated. A pair of paradorsal setae on segments 4, 6, and 8 and other pair of twin paraventral setae on segments 3–7. Lateral terminal spines very long, more than 40% of trunk length. Males without big adhesive tubes on the sternal plates of the second segment (Figs. 3, 4a, b).

Description

Holotype, adult female (Fig. 3). All dimensions and measurements for the examined specimens are summarized in Table 4. The distribution of cuticular trunk structures is summarized in Table 5.

The introvert of the specimens examined was retracted or not extended enough to allow full description.

Neck With 4 dorsal and 2 ventral placids (Fig. 5b).

Segment 1 Anterior dorsal margin of segment serrulated. Anterior lateral margins of tergal plate projecting into horn-like structures (Fig. 4e). Posterior margin forming a free flap that partially overlaps the next segment. The free flap is striated longitudinally, a feature corresponding to the "Knöpfchenreihen" of Zelinka (1928). These structures are in fact small cuticular pillars as shown with TEM by Neuhaus (1993: Fig. 13) (Fig. 5b). Minute pectinate fringe. Two episternal plates and a trapezoidal midsternal plate (Fig. 5a). Episternal plates with sensory spots in ventromedial and ventrolateral positions, and with a muscle scar in ventromedial position. Tergal plate with three pairs of sensory spots (two subdorsal and one laterodorsal pairs), one pair of paralateral setae adjacent to the laterodorsal sensory spot (Figs. 3, 4e), and one pair of subdorsal

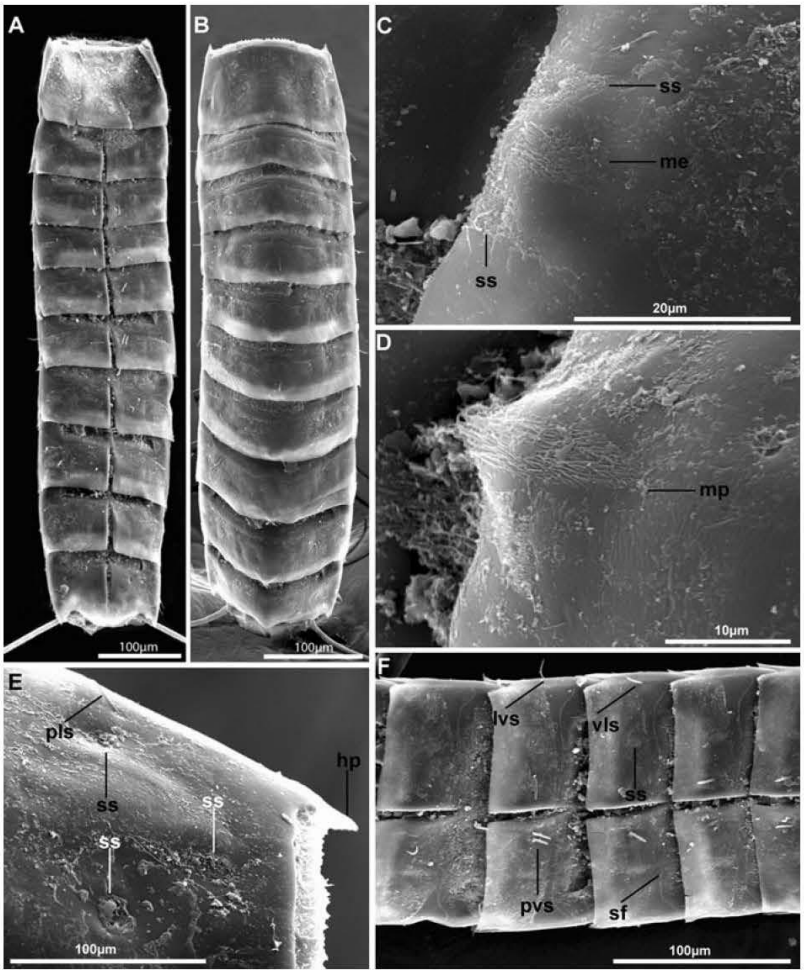

Fig. 4 *Pycnophyes dolichurus* sp. nov., SEM photographs. **a** Male, ventral view. **b** Male, dorsal view. **c** Female; detail of segment 7 showing middorsal elevation near posterior segment margin. **d** Male; detail of segment 9 showing middorsal process near posterior segment margin. **e** Male; detail of anterolateral part of tergal plate of segment 1. **f** Male; segments 3–7, ventral view. *hp* horn-like protrusion, *lvs* lateroventral seta, *me* middorsal elevation, *mp* middorsal process, *pls* paralateral seta, *pvs* paraventral seta, *sf* secondary fringe, *ss* sensory spot, *vls* ventrolateral seta

muscular scars. Segments furthermore with one pair of subcuticular glands (Fig. 5b).

Segment 2 Pachycycli of tergal and sternal plates well developed. Conspicuous secondary fringe of tiny, denticulated cuticular hairs all around the segment. Sternal plates with a pair of ventromedial sensory spots and a pair of ventrolateral setae (Fig. 5c). One pair of longitudinal, ventrolateral muscular scars anterior to the secondary fringe. Tergal plate with smooth posterior margin showing a hairy middorsal elevation, not protruding beyond the segment margin. One pair of paradorsal sensory spots near posterior margin. Prominent paradorsal butterfly-like structures that correspond to subcuticular atria of sensory spots, only visible with DIC optics. One pair of subdorsal twin sensory spots and one pair of lateroventral setae. Males always without paired adhesive tubes that otherwise typically are found on the second segment of the sternal plates in species of *Pycnophyes*.

Segment 3 Sternal plates with a pair of paraventral twin setae and a pair of ventromedial sensory spots (Figs. 4f, 5c). The position of the paraventral twin setae is very consistent in the studied specimens. The only observed variation was displayed as a slight displacement of one of the twin setae in a few specimens. This kind of variation may appear at any segment. Tergal plate with a middorsal elevation, one pair of paradorsal sensory spots near posterior margin with their associated subcuticular atria, one pair of subdorsal sensory spots, one pair of laterodorsal setae, and one pair of lateroventral setae. Secondary fringe similar to fringe on segment 2. One pair of longitudinal ventrolateral muscular scars present anterior to the secondary fringe.

Table 4 *Pycnophyes dolichurus* sp. nov. Measurements (μm) for holotype and means of studied specimens, females and males

Character	Holotype	*n*	Range	Mean	SD	SD ♀–♂
TL	715	♀10	♀649–746	♀699	♀35.87	39.11
		♂4	♂660–769	♂707	♂50.64	
MSW-6	186	♀1	–	–	–	–
SW-10	151	♀1	–	–	–	
SL1	90	♀10	♀84–96	♀90	♀3.10	3.20
		♂4	♂86–93	♀90	♂3.96	
SL2	58	♀10	♀49–63	♀58	♀3.68	3.36
		♂4	♂52–60	♂57	♂3.47	
SL3	54	♀10	♀54–70	♀64	♀4.19	3.80
		♂4	♂58–63	♂60	♂3.12	
SL4	64	♀10	♀59–72	♀67	♀4.81	3.90
		♂4	♂61–66	♂64	♂1.74	
SL5	69	♀10	♀65–76	♀70	♀7.83	7.07
		♂4	♂61–68	♂65	♂3.64	
SL6	74	♀10	♀68–79	♀75	♀3.98	4.94
		♂4	♂64–74	♂68	♂6.38	
SL7	75	♀10	♀75–81	♀78	♀2.00	3.04
		♂4	♂69–80	♂74	♂3.89	
SL8	84	♀10	♀81–88	♀84	♀2.64	2.83
		♂4	♂78–87	♂82	♂3.26	
SL9	88	♀10	♀81–93	♀88	♀4.13	3.93
		♂4	♂81–94	♂87	♂4.49	
SL10	92	♀10	♀89–99	♀96	♀3.59	3.87
		♂4	♂86–99	♂93	♂5.48	
SL11	55	♀10	♀38–55	♀44	♀18.29	15.55
		♂4	♂43–50	♂47	♂3.51	
LTS	320	♀10	♀286–332	♀315	♀13.82	23.43
		♂3	♂243–317	♂288	♂39.83	
%LTS/TL	45%	♀10	♀41–48%	♀45%	♀0.02	0.05
		♂3	♂32–49%	♂42%	♂0.09	

LTS lateroterminal spine, *MSW-6* maximum sternal width (on segment 6), *n* number of measured specimens, *SD* standard deviation, *SL* segment length, *SW-10* standard width (on segment 10), *TL* total length, ♀ female, ♂ male

Segment 4 Tergal and sternal plates same as those on segment 3 but with one pair of paradorsal setae flanking the middorsal elevation. Secondary fringe similar to fringe on segment 2. One pair of longitudinal ventrolateral muscular scars anterior to the secondary fringe.

Segment 5 Sternal plates similar to those on segment 3 except for the presence of a pair of ventrolateral setae (Fig. 4f). Tergal plate same as on segment 3 except for the absence of lateroventral setae. Secondary fringe similar to fringe on segment 2. One pair of longitudinal ventrolateral muscular scars present anterior to the secondary fringe.

Segment 6 Sternal plates same as on segment 3. Tergal plate same as on segment 4 except for the laterodorsal setae that are situated slightly more laterally. Secondary fringe similar to fringe on segment 2. One pair of longitudinal ventrolateral muscular scars present anterior to the secondary fringe.

Table 5 Summary of location of setae and sensory spots in *Pycnophyes dolichurus* sp. nov. arranged by series

Segment	PD	SD	LD	PL	LV	VL	VM	PV
1		ss, ss	ss	se		ss	ss	
2	ss	tss			se	se	ss	
3	ss	ss	se		se		ss	tse
4	se, ss	ss	se		se		ss	tse
5	ss	ss	se			se	ss	tse
6	se, ss	ss	se		se		ss	tse
7	ss	ss	se		se		ss	tse
8	se, ss	ss	se		se	tse	ss	
9	ss	ss	se			se, ss	ss	
10	ss	ss			tse			ss
11					lts	ps(2, m)		

LD laterodorsal, *LV* lateroventral, *PD* paradorsal, *PL* paralateral, *PV* paraventral, *SD* subdorsal, *VL* ventrolateral, *VM* ventromedial

m male condition of sexually dimorphic character, *lts* lateroterminal spine, *ps* penile spine, *se* seta, *ss* sensory spot, *tse* twin setae, *tss* twin sensory spots

Segment 7 Sternal plates same as on segment 3. Tergal plate similar to plate on segment 6, except for the absence of paradorsal setae flanking the middorsal elevation (Fig. 4c). Secondary fringe similar to fringe on segment 2. One pair of longitudinal ventrolateral muscular scars present anterior to the secondary fringe.

Segment 8 Sternal plates without paraventral setae. Segment with a pair of ventromedial sensory spots and one pair of ventrolateral twin setae. The presence of ventrolateral twin setae is very consistent in the animals studied; however, a little variation regarding the relative position of the two setae can be detected in different specimens. Midventral thickenings of ventral pachycycli (Mittelwülste) present near anteromesial margin of segment. Tergal plate same as on segment 6. The middorsal process protruding beyond the segment margin appears bigger and more pointed than the corresponding structures of preceding segments (Fig. 5d). Secondary fringe similar to fringe on segment 2. One pair of longitudinal ventrolateral muscular scars anterior present to the secondary fringe.

Segment 9 Sternal plates without paraventral setae. Sternal plates with pair of ventromedial and ventrolateral sensory spots. The latter is located in twin positions with a pair of ventrolateral setae. Midventral thickenings of ventral pachycycli (Mittelwülste) present near anteromesial margin of segment. One pair of muscular scars, visible only with LM, present in paraventral position. Tergal plate same

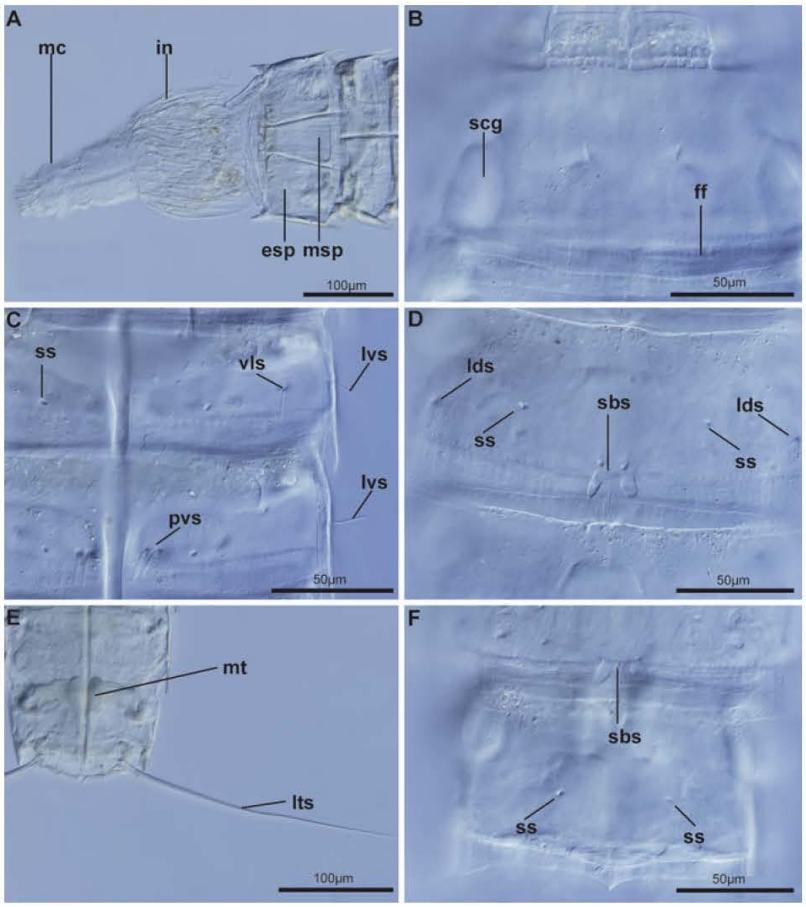

Fig. 5 *Pycnophyes dolichurus* sp. nov., interference contrast (DIC) photographs. **a** Paratype, female; head and segments 1–2, ventral view. **b** Paratype, female; segment 1, dorsal view. **c** Paratype, female; segments 2–3, ventral view. **d** Paratype, female; segment 8, dorsal view. **e** Allotype, male; segments 9–11, ventral view. **f** Paratype, female; segments 9–10, dorsal view. Abbreviations: *esp* episternal plate, *ff* free flap, *in* introvert, *lds* laterodorsal seta, *lts* laterotermial spine, *lvs* lateroventral seta, *mc* mouth cone, *msp* midsternal plate, *mt* anteromesial thickenings of ventral pachycycli (Mittelwülste), *pvs* paraventral seta, *sbs* subcuticular structure (atria), *scg* subcuticular gland, *ss* sensory spot, *vls* ventrolateral seta

as on segment 7, except for the absence of lateroventral setae and the presence of a protruding middorsal process that appears more developed and pointed than on segment 8 (Fig. 4d). Secondary fringe similar to fringe on segment 2. One pair of longitudinal ventrolateral muscular scars present anterior to the secondary fringe.

Segment 10 Sternal plates with a pair of paraventral sensory spots near the posterior margin of segment. Mid-ventral thickenings of ventral pachycycli (Mittelwülste) present near anteromesial margin of segment (Fig. 5c). Tergal plate with one pair of subdorsal sensory spots and one pair of twin lateroventral setae. Laterodorsal setae not present. Posterior margin pointed but without middorsal elevation and with a pair of paradorsal sensory spots (Fig. 5f). Secondary fringe similar to fringe on segment 2. One pair of longitudinal ventrolateral muscular scars anterior to the secondary fringe.

Segment 11 Lateral terminal spines very long, almost half of the trunk length (Fig. 5e). Males with two pairs of penile spines near anterior segment margin (Fig. 3c).

Taxonomic account

Pycnophyes aulacodes sp. nov.

Order Homalorhagida Zelinka, 1896
Suborder Homalorhagae Zelinka, 1896
Family Pycnophyidae Zelinka, 1896
Genus *Pycnophyes* Zelinka, 1907

Examined material

A total of 36 specimens (14 males and 22 females) were examined with DIC and 6 additional specimens (3 males and 3 females) with SEM. The specimens were collected in Ría de Ares and Ría de La Coruña, NW Spain; Blanes, NE Spain and Garrucha and Denia, E Spain. The type series includes the holotypic adult female (locality: Ría de Ares, station no. 070627.1, position: 43° 25.064′N, 008° 16.558′W, date 2007/06/27), allotypic adult male (locality: Ría de Ares, station no. 080404.3; position: 43° 25.400′N,

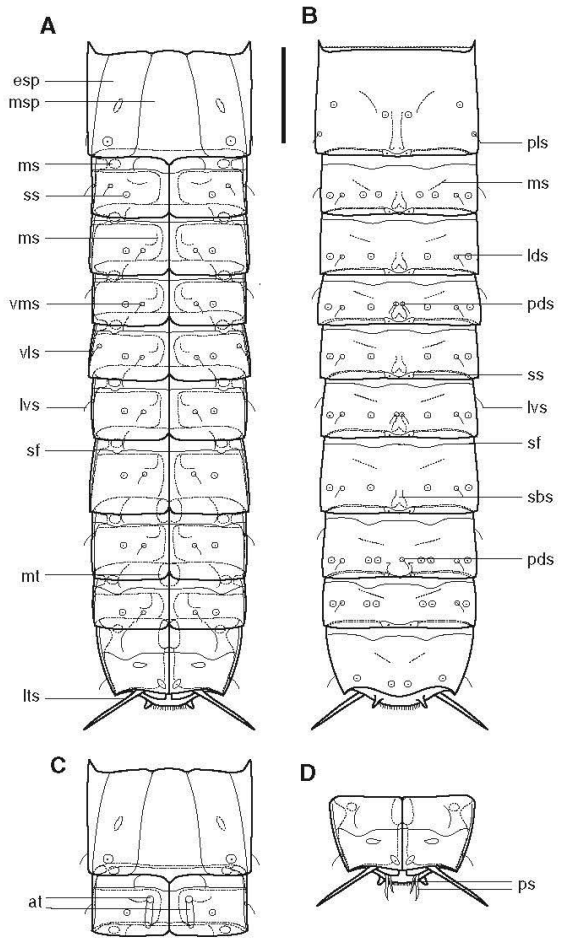

Fig. 6 *Pycnophyes aulacodes* sp. nov. **a** Female, ventral view. **b** Female, dorsal view. **c** Male, ventral view of segments 1 and 2. **d** Male, ventral view of segments 10 and 11. Scale bar: 100 µm. Abbreviations: *at* adhesive tube, *esp* episternal plate, *lds* laterodorsal seta, *lts* laterotermiinal spine, *lvs* lateroventral seta, *mds* middorsal seta, *ms* muscular scar, *msp* midsternal plate, *mt* anteromesial thickenings of ventral pachycycli (Mittelwülste), *pds* paradorsal seta, *pls* paralateral seta, *ps* penile spine, *sbs* subcuticular structure (atria), *sf* secondary fringe, *ss* sensory spot, *vls* ventrolateral seta, *vms* ventromedial seta

008° 20.769′W; date 2008/04/04), and 10 paratypes, 5 males and 5 females from the same locality as the holotype. All types of specimens are mounted in Fluoromount-G® and deposited at the Zoological Museum, University of Copenhagen under accession numbers ZMUC KIN-442 (holotype), ZMUC KIN-443 (allotype), and ZMUC KIN-444 to KIN-453 (paratypes). Additional specimens remain in the personal collection of the first author.

Table 6 *Pycnophyes aulacodes* sp. nov. Measurements (µm) for holotype and means of studied specimens, females and males

Character	Holotype	*n*	Range	Mean	SD	SD♀–♂
TL	713	♀6	♀662–806	♀736	♀53.01	47.04
		♂5	♂727–808	♂775	♂30.70	
MSW-7	171	♀1	–	–	–	–
SW-10	168	♀1	–	–	–	
SL1	112	♀6	♀97–115	♀10	♀6.95	6.63
		♂5	♂107–120	♂113	♂6.52	
SL2	71	♀6	♀48–72	♀65	♀8.67	7.84
		♂5	♂66–79	♂72	♂5.22	
SL3	74	♀6	♀60–77	♀70	♀5.95	5.38
		♂5	♂70–79	♂75	♂3.93	
SL4	75	♀6	♀66–76	♀73	♀3.76	4.62
		♂5	♂66–79	♂72	♂5.93	
SL5	76	♀6	♀67–76	♀74	♀3.54	5.25
		♂5	♂68–79	♂76	♂7.10	
SL6	79	♀6	♀70–87	♀79	♀5.67	4.97
		♂5	♂75–87	♂80	♂4.64	
SL7	85	♀6	♀71–92	♀82	♀7.10	6.68
		♂5	♂76–92	♂84	♂6.72	
SL8	87	♀6	♀75–88	♀83	♀4.60	4.45
		♂5	♂83–92	♂87	♂3.36	
SL9	86	♀6	♀72–87	♀83	♀5.65	5.12
		♂5	♂84–90	♂88	♂2.58	
SL10	85	♀4	♀83–100	♀91	♀7.75	5.36
		♂5	♂88–96	♂92	♂3.41	
SL11	17	♀1	–	♀17	–	–
		♂0	–		–	
LTS	74	♀6	♀74–93	♀80	♀6.57	7.28
		♂3	♂86–89	♂82	♂10.05	
%LTS/TL	10%	♀6	♀10–12%	♀11%	♀0.01	0.01
		♂3	♂9–11%	♂10%	♂0.01	

LTS laterotermiinal spine, *MSW-7* maximum sternal width (on segment 7), *n* number of measured specimens, *SD* standard deviation, *SL* segment length, *SW-10* standard width (on segment 10), *TL* total length, ♀ female, ♂ male

Etymology

The species name, *aulacodes*, is masculine, derived from Greek *aulakodes*, meaning "furrowlike", and refers to the prominent and slit-like subdorsal muscular scars.

Diagnosis

Pycnophyes with middorsal elevations present on segments 1–9. Paradorsal setae present on segments 4 and 6. Tergal anterior margin of segment 1 finely denticulated. Without lateroventral setae on segments 3, 5, 7, and 9. Conspicuous

Table 7 Summary of location of setae and sensory spots in *Pycnophyes aulacodes* sp. nov

Segment	PD	SD	LD	PL	LV	VL	VM	PV
1	ss, ss		ss	se		ss		
2	ss	tss	se, ss		se	se(f)	ss, at(m)	
3	ss	ss	se, ss				se, ss	
4	se, ss	ss	se, ss		se		se, ss	
5	ss	ss	se, ss			se	se, ss	
6	se, ss	ss	se, ss		se		se, ss	
7	ss	ss	se, ss				se, ss	
8	se(1), ss	tss	se, ss		se		se, ss	
9	ss	tss	se, ss				se, ss	
10	ss		ss		se			ss
11					lts	ps(2, m)		

LD laterodorsal, *LV* lateroventral, *PD* paradorsal, *PL* paralateral, *PV* paraventral, *SD* subdorsal, *VL* ventrolateral, *VM* ventromedial

at adhesive tube, *f* female condition of sexually dimorphic character, *lts* laterolateral spine, *m* male condition of sexually dimorphic character, *se* seta, *ss* sensory spot, *tss* twin sensory spots, *(1)* only one unpaired cuticular structure

oblique, groove-like muscular scars in the subdorsal position, and crescentic muscular scars in the paraventral position present on all segments (Figs. 6, 8 b–f).

Description

Holotype, adult female. All dimensions and measurements of the examined specimens are summarized in Table 6. The distribution of cuticular trunk structures is summarized in Table 7.

Neck Placids could not be observed.

Segment 1 Anterior margin of tergal plate serrated. Lateral margins of tergal plate extend into horn-like structures. The posterior dorsal segment margin free, covering the anterior edge of the following segment and overlaps some mucous gland openings (Fig. 7e). This area often appears filled with a mixture of mucus and organic debris, a picture repeated for all trunk segments. Pectinate fringe inconspicuous in the dorsal region but more developed in the

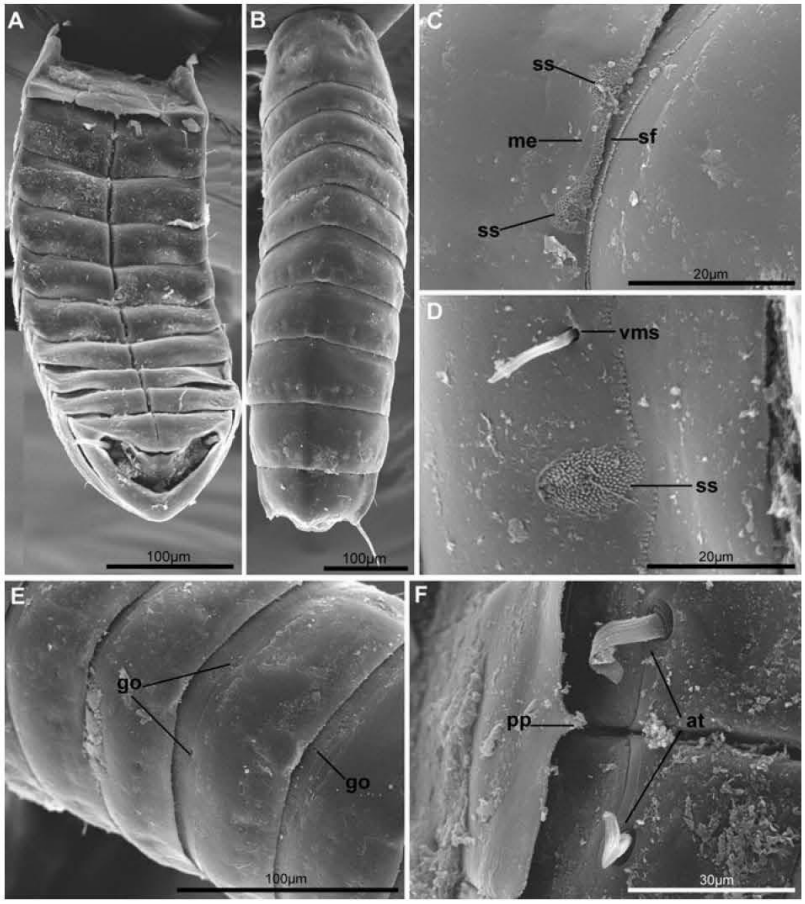

Fig. 7 *Pycnophyes aulacodes* sp. nov., SEM photographs. **a** Male, ventral view. **b** Male, dorsal view. **c** Female; detail showing middorsal position on segments 2–3. **d** Male; segment 5, detail of ventromedial position on left sternal plate. **e** Male; segments 4–7, dorsal view. **f** Male; segment 1–2, ventral view. *at* adhesive tube, *go* gland opening, *me* middorsal elevation, *pp* pointed projection, *sf* secondary fringe, *ss* sensory spot, *vms* ventromedial seta

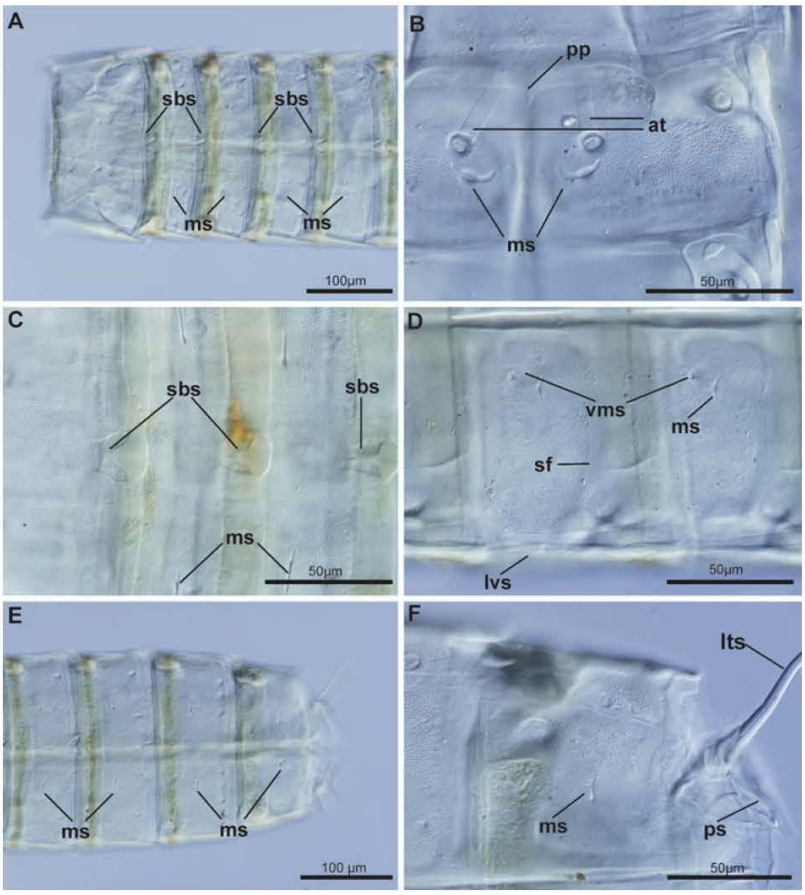

Fig. 8 *Pycnophyes aulacodes*, sp. nov., interference contrast (DIC) photographs. **a** Allotype, male; segments 1–5, dorsal view. **b** Paratype, male; segments 2–3, ventral view. **c** Paratype, female; segments 1–4, dorsal view. **d** Holotype, female; right sternal plates of segments 7–8, ventral view. **e** Paratype, male; segments 7–11, ventral view. **f** Paratype, male; segments 10–11, ventral view. *at* adhesive tube, *lts* laterotermial spine, *lvs* lateroventral seta, *ms* muscular scar, *pp* pointed projection, *ps* penile spine, *sbs* subcuticular structure (atria), *sf* secondary fringe, *vms* ventromedial seta

ventral region. Ventral side with two episternal plates and a trapezoidal midsternal plate. The midsternal plate shows a midventral, pointed, posteriorly orientated projection (Figs. 7f, 8b). Each episternal plate with a conspicuous ventrolateral sensory spot near the posterior margin and a ventromedial muscular scar in the middle region of plate. Tergal plate with a pair of paralateral setae and two pairs of sensory spots, one paradorsal and near the groove-like muscular scars and one laterodorsal pair. Additionally, there is a small middorsal elevation that never surpasses the posterior margin of the segment, with a pair of paradorsal sensory spots near its posterior margin. Prominent paradorsal butterfly-like structures that correspond to subcuticular atria of sensory spots, only visible with DIC optics (Fig. 8a, c).

Segment 2 Sternal plates with a pair of crescentic paraventral muscular scars and a pair of ventromedial sensory spots (Fig. 8b). Females furthermore with a pair of ventrolateral setae. Pectinate fringe strongly reduced, only visible with SEM. Tergal plate with a pair of lateroventral and laterodorsal setae. Subdorsal twin sensory spots and one pair of laterodorsal sensory spots close to the laterodorsal setae are present. Middorsal elevation with a pair of paradorsal, marginal sensory spots (Fig. 8c). Subcuticular structure similar to the one on segment 1. A pair of subdorsal groove-like, oblique muscular scars (Fig. 8c) is present. Males always with one pair of big, adhesive tubes located ventromedially on the sternal plates (Fig. 6c). Secondary fringe near anterior margin, parallel to the segment edge with two subdorsal and two ventromedial indentations (Fig. 8b).

Segment 3 Sternal plates with a pair of paraventral crescentic muscular scars and a pair of ventromedial setae near more lateral sensory spot (Fig. 8b). Tergal plate with middorsal elevation and associated structures similar to those on segment 2, a pair of subdorsal groove-like muscular scars, a pair of laterodorsal setae, and a pair of sensory spots in paradorsal, subdorsal, and laterodorsal positions. Secondary fringe same as fringe on segment 2 (Fig. 7c).

Segment 4 Tergal and sternal plates same as on segment 3, except for the presence of a pair of paradorsal setae in the middorsal elevation and a pair of lateroventral setae. Secondary fringe as fringe on segment 3.

Segment 5 Sternal plates similar to those on segment 3 except for the presence of a pair of ventrolateral setae. Tergal plate same as on segment 3 (Fig. 7d).

Segment 6 Sternal and tergal plates same as those on segment 4.

Segment 7 Sternal and tergal plates same as those on segment 3 (Fig. 8d, e).

Segment 8 Sternal plates same as those on segment 3. One pair of anteromesial thickenings of ventral pachycycli (Mittelwülste) present near anterior segment margin. Tergal plate same as on segment 2 except for the presence of an unpaired paradorsal seta in the right side of middorsal elevation. Middorsal elevation wider than those on the previous segments.

Segment 9 Sternal plates same as those on segment 3, but in addition with one pair of anteromesial thickenings of ventral pachycycli (Mittelwülste) near anterior margin of segment. Tergal plate same as on segment 8 but without lateroventral and paradorsal setae. Middorsal elevation even wider and less conspicuous than the one on the previous segment.

Segment 10 Sternal plates with a pair of droplet-shaped muscular scars and a pair of paraventral sensory spots near the posterior segment margin (Fig. 8f). Anteromesial thickenings of ventral pachycycli (Mittelwülste) present near anterior margin. Tergal plate with a pair of groove-like muscular scars, a pair of paradorsal and laterodorsal sensory spots, and a pair of lateroventral setae. Middorsal elevation not present.

Segment 11 With lateral terminal spines. Males with two pairs of penile spines (Figs. 6d, 8f).

Discussion

Taxonomy

A commonly used diagnostic trait for species of the genus *Pycnophyes* is the presence and appearance of the middorsal—(Higgins 1983) or spinose processes, following the terminology established by Higgins (1983) and summarized by Sørensen and Pardos (2008). The middorsal process is a longitudinal, keel-like protruding edge located in the middorsal line of the segment. It may or may not surpass the posterior margin of the segment and hence form a free tip. Two types are usually recognized: obtuse/rounded ones or pointed/horned ones. The two types are not easily distinguished from each other in practice. Here, we identify the obtuse/rounded type with a "middorsal elevation" that never surpass the posterior edge of the segment and the pointed/horned type with a "middorsal process" that surpasses the posterior segment edge. It should be stressed that both types of processes can be present in the same individual but on different segments. Regarding this trait, reported very confusingly in the literature, *Pycnophyes dolichurus* sp. nov. shows both middorsal processes (pointed and protruding) and middorsal elevations (rounded, not protruding), whereas *P. aulacodes* sp. nov. has only middorsal elevations.

Pycnophyes dolichurus sp. nov. has middorsal elevations on segment 2 and the following five segments. On segments 8 and 9, these elevations are modified into middorsal processes. All middorsal elevations and processes are equipped with paradorsal, subcuticular, butterfly-like atria. Diagnostic characters for the species include a tergal anterior margin of the first trunk segment being strongly denticulated, a pair of paradorsal setae on segments 4, 6, and 8, and pairs of twin paraventral setae on segments 3 to 7. Furthermore, its lateral terminal spines are conspicuously long, more than 40% of trunk length. A feature not very common in the genus is the absence of the characteristic big adhesive tubes on the sternal plates of segment 2 in males. This last trait is only shared with three other species in the genus, namely *P. ecphantor* Higgins, 1983, *P. egyptensis* Higgins, 1966, and *P. longicornis* Higgins, 1983, but all of them have a different distribution of setae: *P. ecphantor* and *P. egyptensis* have middorsal setae and *P. longicornis* has setae present in females only, whereas *P. dolichurus* sp. nov. has no middorsal but paradorsal setae in different segments (Higgins 1966, 1983). Two additional species, *P. mokievskii* Adrianov, 1995, and *P. spitsbergensis* Adrianov 1995, have confusing descriptions, since the adhesive tubes on segment 2 are reported in the text but not illustrated in drawings (see Adrianov 1995, Figs. 7, 11, and 13).

Pycnophyes dolichurus sp. nov. is the species of the genus with the longest lateral terminal spines described so far. Other species, including *P. longicornis*, *P. chiliensis* Lang, 1953, and *P. canadensis* Higgins and Korczynski, 1989 also have extraordinary long lateral terminal spines—a character that enables the observer to identify these species of *Pycnophyes* easily. However, none of them present a TL/LTS proportion similar to *P. dolichurus* sp. nov. (Lang 1953; Higgins 1983; Higgins and Korczynski 1989). From these, the closest species in terms of lateral terminal spine length is *P. longicornis* (37.7% of TL in males). However, this species shows a transverse mark at each lateral terminal spine about three-fourths from its base. Such marks are not present in *P. dolichurus* sp. nov.

The presence of paired paradorsal setae in *Pycnophyes dolichurus* sp. nov. is shared with *P. parasanjuanensis* Adrianov and Higgins, 1996, *P. greenlandicus* Higgins and Kristensen, 1988, *P. faveolus* (in Brown 1985, see Adrianov and Malakhov 1999) and *P. kielensis* Zelinka, 1928. However, *P. dolichorus* sp. nov. possesses paradorsal setae on segments 4, 6, and 8, whereas such setae are found on segment 6 only in *P. parasanjuanensis* and on segment 8 in *P. greenlandicus* (Higgins and Kristensen 1988; Adrianov and Higgins 1996). Also, *P. faveolus* and *P. kielensis* have paradorsal setae, but in these species the setae are not only restricted to segments 4, 6, and 8. Hence, *P. faveolus* has paradorsal setae on five segments (Brown 1985), whereas such setae are present on at least eight segments in *P. kielensis* (not reported by Zelinka 1928), but see Neuhaus (1993) and Adrianov and Malakhov (1999).

Moreover, *Pycnophyes dolichurus* sp. nov. shows unique characters that are not present in any other species of the genus, such as one pair of twin setae on every sternite of segments 3–7.

Pycnophyes aulacodes sp. nov. has middorsal elevations with subcuticular butterfly-like atria on segment 1 and following segments, and on segments 4 and 6 also a pair of adjacent paradorsal setae is present. Tergal anterior margin of segment 1 is minutely denticulated, and lateroventral setae are lacking on segments 3, 5, 7, and 9.

Pycnophyes aulacodes sp. nov. is easily recognized by the presence and appearance of muscular scars on all segments. The scars are groove-like on the tergal plates and appear crescentic on the sternal plates of segments 2–9. It should be noted that these cuticular marks show some variation among specimens, being even more conspicuous in older animals, where the cuticle becomes thicker with age. Furthermore, some species descriptions dating back to the middle of the twentieth century do not document this feature. Hence, this character should be used with caution when comparing species and specimens, and a revision and redescription of many species in the genus would be needed in order to come to sound taxonomic statements.

There are some species in which similar groove-like and crescentic muscular scars have been reported, including *P. beaufortensis* Higgins, 1964, *P. egyptensis*, *P. corrugatus* Higgins, 1983, *P. ecphantor* and *P. neuhausi* Martorelli and Higgins, 2004 (Higgins 1964b, 1966, 1983; Martorelli and Higgins 2004). However, *P. beaufortensis*, *P. corrugatus* and *P. ecphantor* have no middorsal elevation on segment 1. Moreover, none of these three species have paradorsal setae, although *P. corrugatus* and *P. ecphantor* show middorsal setae 2–9 and 2, 4–8, respectively. *P. egyptensis* differs from *P. aulacodes* sp. nov. by having no middorsal elevations or processes, and although middorsal setae are present (4–10, see Higgins 1966), they never possess paired paradorsal setae. *Pycnophyes neuhausi* has a middorsal elevation on segment 1, but the posterior margin of this segment extends posteriorly well over the following segment. Moreover, *P. aulacodes* sp. nov. and *P. neuhausi* differ in their distributions of ventral setae (two pairs of setae on the sternal plates in segments 3–5 of *P. neuhausi* and only one in *P. aulacodes*) and in the absence of middorsal setae in *P. neuhausi*.

The presence of paradorsal setae on the segments 4 and 6 in *Pycnophyes aulacodes* sp. nov. is shared with *P. dolichurus* sp. nov., and as stated above, *P. parasanjuanensis*, *P. greenlandicus*, *P. faveolus,* and *P. kielensis* (Zelinka 1928; Brown 1985; Higgins and Kristensen 1988; Adrianov and Higgins 1996; Adrianov and Malakhov 1999). However, both *P. parasanjuanensis* and *P. greenlandicus* show paradorsal setae on one segment only, whereas *P. faveolus* and *P. kielensis* show paradorsal setae on more segments or the setae have a different distribution. Hence, *P. aulacodes* sp. nov. can easily be distinguished from any known species in the genus.

Character evaluation

The main source of mistakes in the determination of specimens in the genus *Pycnophyes* is the triangular shape of the animals in cross section, which makes it difficult to distinguish and locate some diagnostic characters using LM. Due to this, the ability to make accurate observations of the dorsal or ventral regions of the specimens depends on the specimen's orientation (dorsal or ventral up) on the glass slide. In addition, a further consequence is the difficulty to locate and discriminate laterodorsal structures because they often appear too close the lateral margins of the specimen.

These problems are best solved by the use of SEM, which allows observation from any point of view. However, SEM is restricted to provide information about cuticular surface characters, whereas LM allows observation of subcuticular structures. Hence, the optimal examination of a species is obtained when the two techniques are used in combination.

Finally, there are some characters used in identification keys that are ambiguous and lead to confusion. For example, the determination of whether a middorsal process is more or less rounded or pointed, or the exact consideration of a middorsal process that exceeds or not the segment margin—a feature that may vary according of the contraction or squeezing state of the specimen. Those are very subjective characters, and their accurate evaluation depends mostly on the researcher view and ability. Unfortunately, most, if not all, keys for homalorhagid kinorhynchs make frequent use of those characters and features. We hope that the description of the new species presented here and other to come in a near future will enable the preparation of completely new, unambiguous keys for homalorhagid kinorhynchs.

Faunistic remarks

Information on kinorhynch distribution is scarce. The available data refer only to the species found in sampling localities randomly selected by several authors throughout a long timespan, whereas only few expanded areas or long coast lines have been object of systematic sampling campaigns. Hence, the distribution patterns of most kinorhynch species are not fully understood. This paper is part of a long-term study along the coasts of Spain, facing the Atlantic Ocean to the north and west and the Mediterranean to the east.

The first recordings of species of the genus *Pycnophyes* along the Spanish coasts were surprising. Since species of the genus not previously have been recorded from Iberian Peninsula, it was expected that the specimens collected in the sampled localities, from Galicia (NW) to the Costa Brava (NE), including Almería (SE) and the Levantine coast (E) would, at least in part, be conspecific with those that had been reported from localities close to the Iberian Peninsula. In fact, and as stated previously, 11 species of this genus have been recorded from the Atlantic Ocean and the Mediterranean Sea (Reinhard 1881; Southern 1914; Zelinka 1928; Reimer 1963; Higgins 1983; Adrianov and Malakhov 1999).

However, none of the previously recorded species were encountered among the specimens collected for the present study. This excluding distribution may be due to two reasons. First, the genus is most probably more diverse in European waters than we know currently; hence, the new species described in the present contribution will probably appear at other Atlantic and Mediterranean localities as well. Secondly, the meiofauna distribution and in particular the kinorhynch distribution is strongly patched (Gray and Rieger 1971; Mclachlan 1978; Findlay 1981; Higgins 1988). As a consequence, it is highly probable that the present sampling campaign, although more intense than other sampling around Europe, has not revealed all existing species around the Iberian Peninsula. Therefore, it should be expected that future samplings in the same or in other localities will demonstrate the presence of different species of *Pycnophyes* that already have been recorded elsewhere in Europe.

Pycnophyes dolichurus sp. nov. appeared only in the Ría de Ares, Galicia, NW Spain, whereas *P. aulacodes* sp. nov. was found on the same locality but also at the nearby Ferrol and La Coruña, as well as in the Mediterranean localities of Blanes, Garrucha and Denia, NE, SE, and E Spain. This geographical distribution suggests that *P. dolichurus* sp. nov. has a more restricted distribution, whereas *P. aulacodes* sp. nov. probably occurs all along the Iberian coast. Nevertheless, more intense sampling campaigns should be carried out to confirm these rather different biogeographical distributions.

In addition, the two species also appear to differ in their sediment preferences. From our results, none of them appeared in shell gravel or amphioxus sand (samples not shown in Table 3), and *P. dolichurus* sp. nov. never appeared in muddy sediments. *P. dolichurus* sp. nov. is found in sandy sediments, and mostly in the fine sand. *P. aulacodes* sp. nov. prefers silty and sandy sediments, apparently with a preference for the more muddy ones.

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Pycnophyes almansae sp. nov. and *Pycnophyes lageria* sp. nov., two new homalorhagid kinorhynchs (Kinorhyncha, Homalorhagida) from the Iberian Peninsula, with special focus on introvert features

Nuria Sánchez, María Herranz, Jesús Benito, Fernando Pardos

RESUMEN: ***Pycnophyes almansae* sp. nov. y *Pycnophyes lageria* sp. nov., dos nuevos kinorrincos homalorrágidos (Kinorhyncha, Homalorhagida) de las costas españolas, con especial atención en los caracteres del introverto.** – Se describen dos nuevas especies de kinorrincos homalorrágidos del género *Pycnophyes*, *Pycnophyes almansae* sp. nov. y *Pycnophyes lageria* sp. nov., recolectados en las costas españolas del océano Atlántico y del mar Mediterráneo, mediante microscopía electrónica de barrido y microscopía de interferencia de contraste (SEM). *Pycnophyes almansae* sp. nov. se reconoce por la presencia de elevaciones mediodorsales en los segmentos 2-9, pares de sedas paradorsales en los segmentos del 3-9 y una única seda paradorsal en el segmento 2, combinado con la presencia de prominentes marcas musculares en forma de ranura en los segmentos en posición subdorsal y ventromedial en los segmentos 1-10 y un patrón reticular de pliegues cuticulares en las zona ventrolateral de los segmentos 2-10. *Pycnophyes lageria* sp. nov.se diferencia por la ornamentación en el margen dorsal anterior del segmento 1, la cual recuerda a las gotas de cera de una vela, así como por un áreas cuticulares deprimidas en la región ventral del segmento 10, combinado con la presencia de áreas sensoriales similares a los *Nanaloricus flosculi*, y sin estructuras mediodorsales ni sedas medio dorsales o paradorsales. Por primera vez, en éste estudio se incluye una descripción detallada y el mapeo de un introverto en una especie de *Pycnophyes* basándonos en datos de SEM, la cual es comparada con la información disponible en el filo.

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ORIGINAL ARTICLE

Pycnophyes almansae sp. nov. and *Pycnophyes lageria* sp. nov., two new homalorhagid kinorhynchs (Kinorhyncha, Homalorhagida) from the Iberian Peninsula, with special focus on introvert features

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Abstract
Two new species of homalorhagid kinorhynchs of the genus *Pycnophyes*, *Pycnophyes almansae* sp. nov. and *Pycnophyes lageria* sp. nov., are described from the Atlantic and Spanish waters of the Mediterranean Sea, using scanning electron microscopy and differential interference contrast microscopy. *Pycnophyes almansae* sp. nov. is recognized by the presence of middorsal elevations on segments 2–9, pairs of paradorsal setae on segments 3–9 and a single paradorsal seta on segment 2, combined with conspicuous groove-like muscular scars in the subdorsal and ventromedial position on segments 1–10 and a reticulate pattern of cuticular wrinkles in ventrolateral areas on segments 2–10. *Pycnophyes lageria* sp. nov. is distinguished by its ornamented anterior margin of segment 1, resembling candle wax drops; and its depressed cuticular areas on the ventral region of segment 10, combined with the presence of sensory spots similar to *Nanaloricus* flosculi, and without middorsal structures or middorsal or paradorsal setae. For the first time, this study presents a detailed description and mapping of the introvert in a species of *Pycnophyes* based on SEM data, which is compared with the information available on the phylum.

Key words: *Meiofauna, Pycnophyidae, taxonomy, Atlantic, Mediterranean*

Introduction

Until 2010, studies of Iberian kinorhynchs have been limited to the cyclorhagid *Echinoderes*. *Echinoderes dujardini* Claparède, 1863 was reported from Mallorca, the Balearic Islands and the Mediterranean Iberian coast (Pagenstecher 1875), but the identity of these recordings needs to be confirmed. However, the species has recently been recorded near Almuñécar (Granada, S. Spain) by Sánchez-Tocino et al. (2011). Since 1998, five new species of *Echinoderes* (see Pardos et al. 1998; G^{a}Ordóñez et al. 2008), one of *Dracoderes* (Sørensen et al. 2012) and one of *Meristoderes* (Herranz et al. 2012) have been discovered and described from the Iberian coast.

Recently, several species of homalorhagid kinorhynchs have been recorded from Spanish waters: *Paracentrophyes quadridentatus* (Zelinka, 1928) from the Cantabric coasts (Sørensen et al. 2010), *Pycnophyes dolichurus* Sánchez et al., 2011 that was described from Ría de Ares, Galicia, NW Spain and *P. aulacodes* Sánchez et al., 2011 from the same locality and the nearby Ría de Ferrol and Ría de La Coruña (Atlantic coasts). The latter species also occurs in the Mediterranean localities of Blanes, Denia and Garrucha, NE, E and SE Spain, respectively (Sánchez et al. 2011). Our current information on kinorhynch biodiversity around the Iberian Peninsula has been addressed recently by Sánchez et al. (2012).

The aim of this study is to describe two additional new species of *Pycnophyes* from the Iberian Peninsula and provide the first detailed description using SEM of the introvert morphology and scalid arrangement from a *Pycnophyes* species. The new species *P. almansae* sp. nov. and *P. lageria* sp. nov. correspond to *P.* sp. 1 and *P.* sp. 2, respectively, in Sánchez et al. (2012).

Materials and methods

Specimens of *Pycnophyes* were collected from different sampling stations along the Spanish coasts. Atlantic sampling localities included Ría de Ferrol, Ría de Ares and Ría de La Coruña (North Galicia,

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La Coruña, NW Spain); Ría de Vigo (South Galicia, Pontevedra, NW Spain); Cádiz Bay (SW Spain) and Algeciras Bay (SW Spain). A single Mediterranean locality was sampled as well: Almuñécar (S Spain) (Figure 1). Sediment samples were taken with a Higgins Meiobenthic Dredge (Higgins 1964, 1988) that collects the upper centimetres of the sediment. Meiofaunal animals with hydrophobic cuticle were extracted from the sediment using the bubbling technique of Higgins (Higgins 1988; Sørensen & Pardos 2008), fixed in 7% formalin and dyed with Rose Bengal to ease sorting. Collecting data are summarized in Table 1.

Kinorhynch specimens were sorted under a dissecting microscope using an Irwin loop and mounted for LM, either on regular slides or on Cobb slides with either Hoyer's medium or Fluoromount-G®, following standard procedures (Sørensen & Pardos 2008).

The specimens were examined and photographed using an Olympus BX51 microscope equipped with differential interference contrast (DIC) optics and an Olympus DP70 camera. Several specimens were prepared for scanning electron microscopy (SEM), through dehydration in a graded series of ethanol, critical-point dried, mounted on aluminium stubs and sputter-coated with gold. These were studied and photographed with either a JEOL JSM-6400 or JEOL JSM-6335F field-emission SEM.

This study follows the terminology and standardization criteria for homalorhagid kinorhynchs developed by Sánchez et al. (2011). Terminology shared by both homalorhagid and cyclorhagid kinorhynchs follows Sørensen & Pardos (2008) and Herranz et al. (2012). The number and distribution of introvert appendages has been mapped both by rings and sectors using polar diagrams first established by Zelinka (1928), standardized by Higgins (1990) and later followed in the literature with no substantial amendments until its last version by Sørensen & Pardos (2008). The description of scalids follows the standards established by Brown (1989) and Neuhaus (2012).

Taxonomy

Order Homalorhagida (Zelinka, 1896)
Suborder Homalorhagae Zelinka, 1896
Family Pycnophyidae Zelinka, 1896
Genus *Pycnophyes* Zelinka, 1907

***Pycnophyes almansae* sp. nov.**
Figures 2–4; Tables II–III

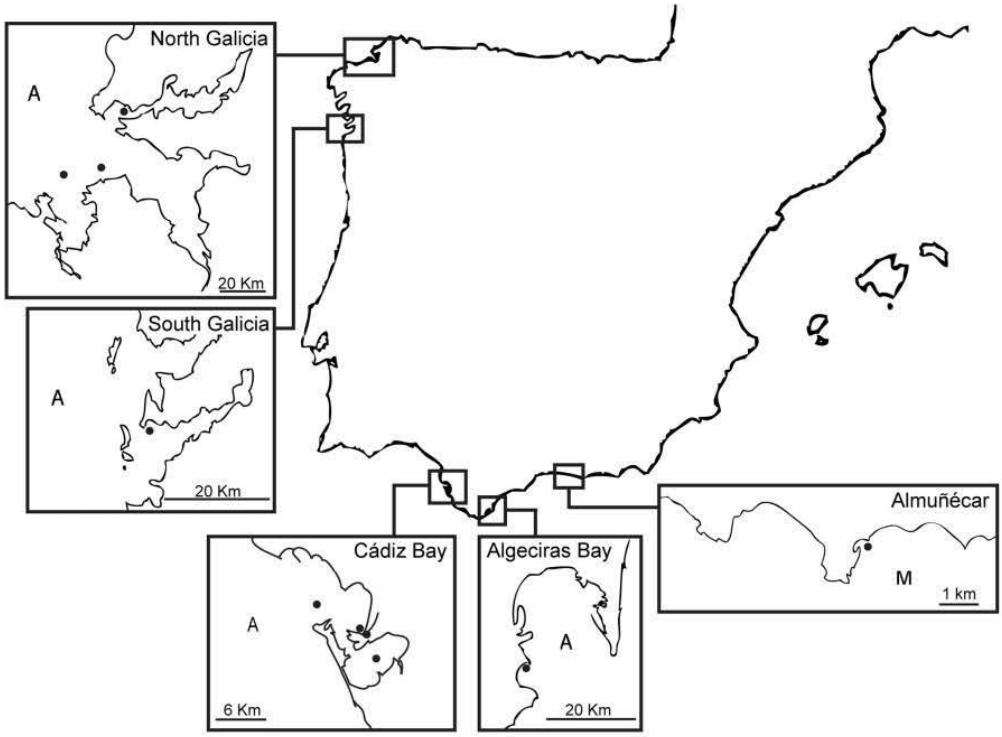


Figure 1. Map of the Iberian Peninsula showing sampling areas and localities. Abbreviations: M, Mediterranean Sea; A, Atlantic Sea.

Table I. Sampling stations referred to in this study.

Date	Locality	Coordinates	Sediment	Depth (m)	*P. almansae* sp. nov.	*P. lageria* sp. nov.
23 September 1993	La Cortadura, Cádiz Bay	36°31′34.95″N 06°13′31.75″W	Mud with *Zostera*	1.5		Paratypes ZMUC KIN-623 to 627
10 November 2011	Cádiz Bay	36°33′45.3″N 06°18′27.0″W	Muddy sand	13		
10 November 2011	Cádiz Bay	36°32.761′N 06°16.268′W	Coarse sand	11	Paratype ZMUC KIN-618	
11 November 2011	Cádiz Bay	36°29.798′N 06°12.871′W	Mud with *Zostera*	1		Holotype ZMUC KIN-621 Allotype ZMUC KIN-622
27 June 2007	Ría de Ferrol, San Cristobo	43°27.887′N 08°18.118′W	Muddy midfine sand	–	Allotype ZMUC KIN-617	
4 April 2008	Ría Coruña	43°24.981′N 08°22.853′W	Fine sand	53		
4 April 2008	Ría de Ares	43°25.400′N 08°20.769′W	Fine sand	45.4	Paratype ZMUC KIN-619	
25 April 2009	Ría de Vigo	42°14.640′N 08°51.487′W	Sand and shell gravel	22.5		
24 July 2010	Almuñécar	36°43′42.70″N 03°43′17.37″W	Coarse sand	18	Holotype ZMUC KIN-616	
7 February 2011	Algeciras Bay	36°05.805′N 05°26.284′W	Fine sand and shell gravel	30	Paratype ZMUC KIN-620	

Material examined

A total of 9 specimens (5 males and 4 females) were examined with DIC, and 2 additional specimens (1 male and 1 female) were examined with SEM. The specimens were collected at 7 localities: Ría de Ferrol, Ría de Ares, Ría de La Coruña and Ría de Vigo, NW Spain (Atlantic); Cádiz Bay, Algeciras (Atlantic) and Almuñécar (Mediterranean), S Spain. The type series includes the holotypic adult male (locality: Almuñécar, 36°43′42.70″N, 3°43′17.37″W, 24 July 2010), allotypic adult female (locality: Ría de Ferrol, 43°27.887′N, 8°18.118′W, 27 June 2007) and 3 paratypes, 2 males and 1 female (one male from Algeciras, one male from Cádiz Bay, female from Ría de Ares, see Table I for detailed sampling locations and coordinates). All type specimens are mounted in Fluoromount-G® and deposited at the Zoological Museum, University of Copenhagen under accession numbers ZMUC KIN-616 (holotype), ZMUC KIN-617 (allotype) and ZMUC KIN-618 to KIN-620 (paratypes). Additional specimens are deposited in the Marine Zoology Laboratory, Dept. of Zoology and Anthropology, Universidad Complutense de Madrid.

Diagnosis

Pycnophyes with middorsal elevations and paradorsal butterfly-like subcuticular atria from sensory spots on segments 2 9. Tergal anterior margin of segment 1 denticulated. With pairs of paradorsal setae on segments 3 9 and a single paradorsal seta on segment 2. Laterodorsal setae on segments 2 8; paralateral ones on segment 1; lateroventral ones on segments 2 4, 6 8, 9 (the latter present in males only) and 10; ventrolateral ones on segments 1, 2 (the latter present in females only), 5 and 10; and ventromedial ones on segments 3 9. Only type 1 sensory spots on all segments. Conspicuous oblique, groove-like muscular scars in the subdorsal position and similar but slightly curved ones in the ventromedial position on segments 1 10 all of them only visible with DIC. Conspicuous reticulate pattern of cuticular wrinkles limited to the anterior ventrolateral areas on segments 2 10. See Figures 2, 3 and 4.

Description

Dimensions and measurements of the examined specimens are summarized in Table II. The distribution of cuticular setae and sensory spots is summarized in Table III.

Introvert and mouth cone. None of the specimens examined had the introvert and mouth cone extended, so data on the number and arrangement of scalids and oral styles are not available.

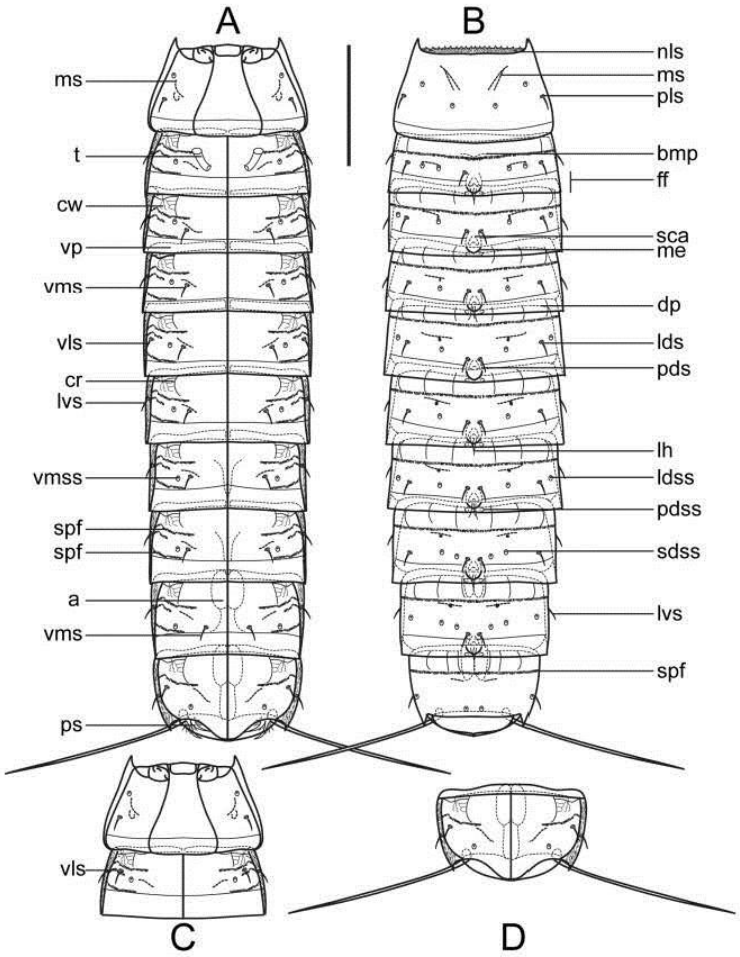


Figure 2. Line art illustrations of *Pycnophyes almansae* sp. nov. (A) Male, ventral view; (B) male, dorsal view; (C) female, segments 1-2, ventral view; (D) female, segments 10 11, ventral view. Scale bar: 100 µm. Abbreviations: a, apodeme, anteromesial thickenings of ventral pachycycli (Mittelwülste); bmp, blunt anterior middorsal projection; cr, cuticular ridge; cw, cuticular wrinkles; dp, dorsal pachycycli; ff, free flap; lds, laterodorsal seta; ldss, laterodorsal sensory spot; lh, 2 4 long hairs over the middorsal elevation; lvs, lateroventral seta; me, middorsal elevation; ms, muscular scar; nls, net-like structure; pds, paradorsal seta; pdss, paradorsal sensory spot; pls, paralateral seta; ps, penile spine; sca, subcuticular atria of sensory spot; sdss, subdorsal sensory spot; spf, secondary pectinate fringe; t, tube; vls, ventrolateral seta; vms, ventromedial seta; vmss, ventromedial sensory spot; vp, ventral pachycycli.

Neck. With 4 dorsal placids and 2 ventral placids. All placids seem robust, hard, with a concave surface. Dorsal placids are of similar size and nearly square in profile. Ventral placids widely rectangular, extending from the lateral margin of the episternal plate to the midventral line. All placids are joined to the anterior edge of segment 1 by one of their wide sides and continue anteriorly with the thin and flexible cuticle of the introvert, leaving a wide nude area behind the introvert. Trichoscalid plates are absent.

Trunk. With 11 segments. First segment with one tergal plate and three sternal plates. Segments 2–11 with one tergal plate and two sternal plates (Figures 2, 3A,B, 4A,B). Cuticle thick throughout the whole trunk and pachycycli well-developed in all segments. Segments 3–10 with four pairs of longitudinal cuticular ridges, namely ventromedial, laterodorsal and two subdorsal ones on the anterior margin (Figures 2, 4A,B). Segment 2 without such subdorsal ridges. Reticulate pattern of cuticular wrinkles limited to the anterior lateral areas of ventral side (Figures 2, 4A,B). Peg and socket joints (tergosternal articulations) clearly present on segments 2 and 3 only. The segment width is fairly constant between segments 3 and 8, with a conventional maximum width at segment 5, but it turns conspicuously

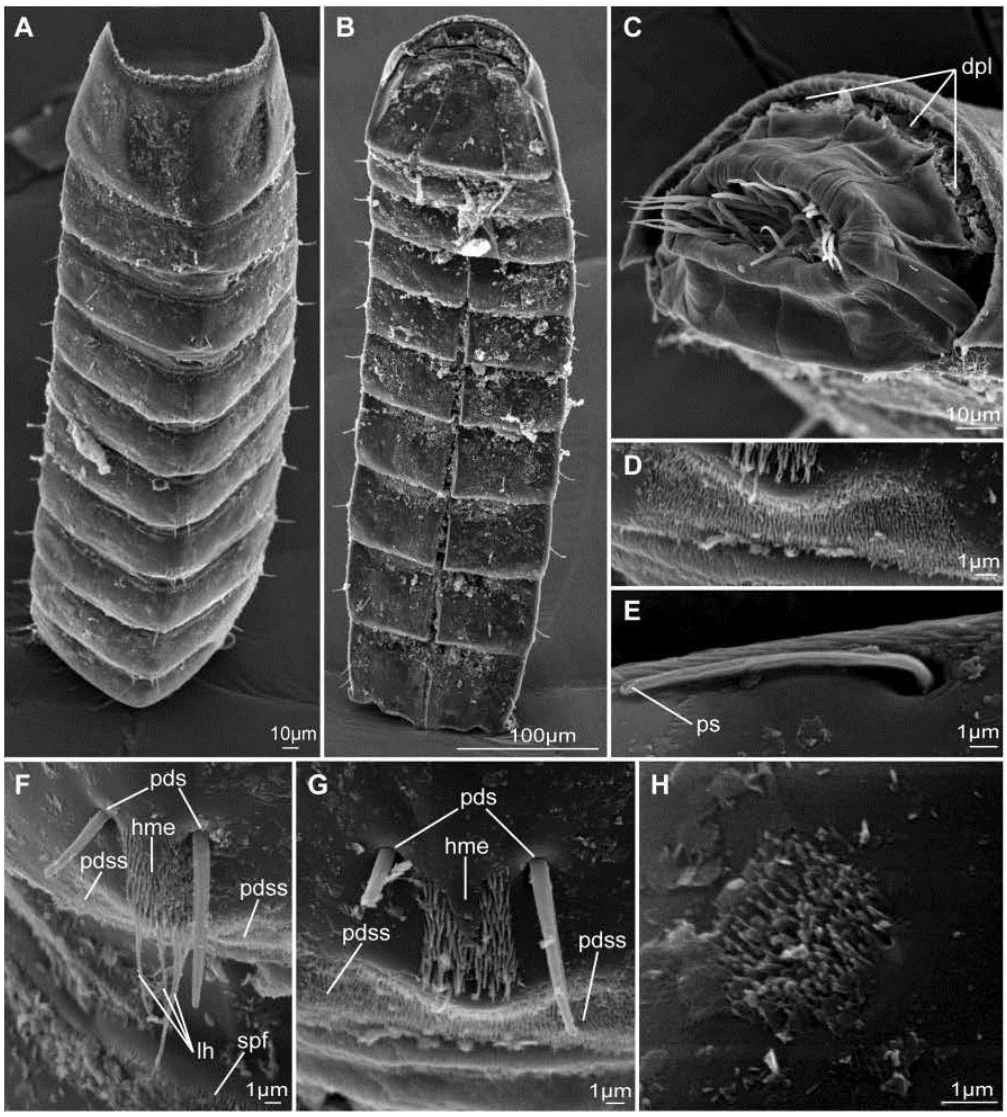


Figure 3. *Pycnophyes almansae* sp. nov., SEM photographs. (A) Male, dorsal view; (B) male, ventral view; (C) neck, laterofrontal view; (D) detail of segment 3 showing paradorsal sensory spot; (E) detail of segment 5 showing ventrolateral seta; (F) detail of segment 7 showing middorsal elevation with long hairs; (G) detail of segment 3 showing middorsal elevation without long hairs; (H) detail of segment 10 showing ventromedial sensory spot. Abbreviations: dpl, dorsal placids; hme, hairy middorsal elevation; lh, 2 4 long hairs over the middorsal elevation; pds, paradorsal seta; pdss, paradorsal sensory spot; ps, pore seta; spf, secondary pectinate fringe.

narrower from segment 9. Tergal plates of segments 2–9 with middorsal elevations, never surpassing the posterior segment edge, and paradorsal butterfly-like subcuticular atria (Figures 2, 3A, 4B). One pair of conspicuous attachment points of dorsoventral muscles present on segments 1–10, visible as oblique, groove-like muscular scars in subdorsal and ventromedial (curved) positions (Figures 2, 4A–D). Posterior margin of tergal and sternal plates (free flap) smooth, overlapping the anterior edge of the following segment (Figures 2, 4A,B). Minute pectinate fringe, only visible with SEM.

Segment 1. Lateral margins of tergal plate project anteriorly into horn-like extensions. Tergal plate with denticulated anterior margin followed by an inconspicuous narrow net-like area. Pairs of conspicuous

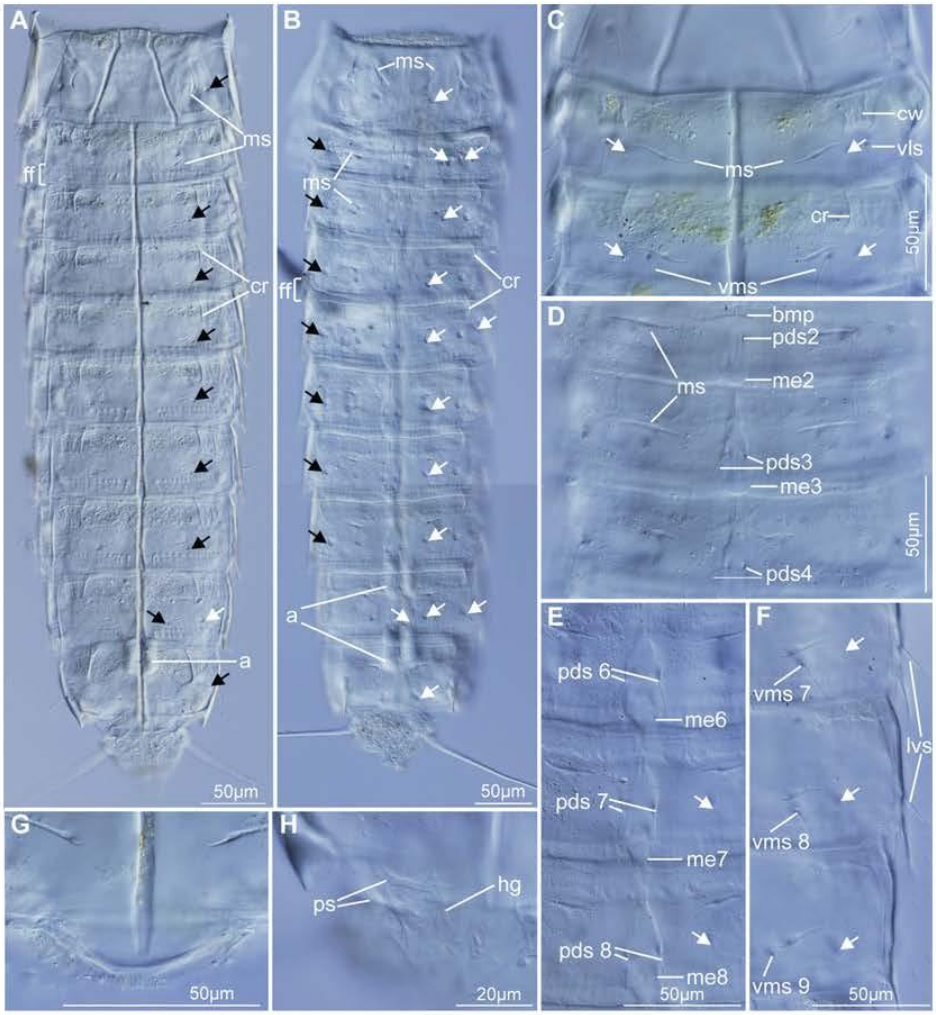


Figure 4. *Pycnophyes almansae* sp. nov., DIC photographs. (A) Male, ventral view, with segment 11 protruding abnormally; (B) male, dorsal view, segment 11 protruding abnormally; (C) male, segments 1–3, ventral view; (D) segments 2–4, middorsal elevation with the paradorsal setae; (E) segments 6–8, middorsal elevation with the paradorsal setae; (F) left side of segments 7–9, ventral view; (G) female, segments 10–11, ventral view, short hairs on tergal plate of segment 11; (H) male, penile spines and tuft of long hairs surrounding the gonopore, ventral view. Abbreviations: a, apodeme, anteromesial thickenings of ventral pachycycli (Mittelwülste); bmp, blunt anterior middorsal projection; cr, cuticular ridge; cw, cuticular wrinkles; ff, free flap; hg, tuft of long hairs surrounding the gonopore; lvs, lateroventral seta; me, middorsal elevation; ms, muscular scar; pds, paradorsal seta; ps, penile spine; vls, ventrolateral seta; vms, ventromedial seta. Sensory spots are marked with white arrows. Setae are marked with black arrows. Digits following labels refer to segment numbers.

oblique, groove-like muscular scars in the subdorsal position in the middle region of the plate (Figures 2B, 4B). One pair of paralateral setae and two pairs of sensory spots in subdorsal and more posteriorly, laterodorsal positions. Ventral side with two episternal plates and a trapezoidal midsternal plate. Each episternal plate with one ventrolateral sensory spot, one ventrolateral seta and a prominent longitudinal muscular scar in the middle region of each plate (Figure 2A).

Segment 2. Tergal plate with a pair of conspicuous oblique, groove-like muscular scars in the subdorsal position (Figure 4D). Hairy middorsal elevation with 2–4 additional long hairs (Figures 2B, 4D; similar to 3D) and a paradorsal butterfly-like structure, only visible with DIC, which corresponds to the subcuticular atria of two posterior paradorsal sensory spots. One pair of laterodorsal and lateroventral setae. Males with single paradorsal seta on the right side of the middorsal elevation, except in one of

Table II. *Pycnophyes almansae* sp. nov. Measurements (μm) for holotype and means of studied specimens, females and males. Abbreviations: LTS, lateroterminal spine; MSW-5, maximum sternal width (on segment 5); n, number of measured specimens; SD, standard deviation; SL, segment length; SW-10, standard width (on segment 10); TL, total length; ♀, female; ♂, male.

Character	Holotype	*n*	Range	Mean	SD	SD ♀–♂
TL	609	3♀	♀558–600	♀575	♀22.59	25.90
		2♂	♂553–609	♂581	♂40.18	
MSW-5	155	1♂	–	–	–	–
SW-10	131	1♂	–	–	–	–
SL1	81	3♀	♀75–78	♀77	♀1.71	1.91
		3♂	♂77–81	♂79	♂1.56	
SL2	56	4♀	♀45–52	♀49	♀2.99	3.35
		3♂	♂56–50	♂52	♂3.47	
SL3	52	4♀	♀47–58	♀53	♀4.26	3.09
		3♂	♂51–52	♂52	♂0.54	
SL4	59	4♀	♀49–61	♀56	♀4.85	3.65
		3♂	♂55–59	♂57	♂1.88	
SL5	65	4♀	♀54–65	♀58	♀4.80	4.72
		2♂	♂61–65	♂63	♂2.74	
SL6	65	4♀	♀56–64	♀60	♀3.25	3.30
		3♂	♂58–65	♂60	♂4.10	
SL7	64	4♀	♀60–67	♀62	♀3.38	2.99
		3♂	♂58–64	♂61	♂2.94	
SL8	67	4♀	♀60–68	♀64	♀3.31	3.20
		3♂	♂60–67	♂63	♂3.78	
SL9	67	4♀	♀61–68	♀64	♀3.40	3.27
		3♂	♂59–67	♂63	♂3.80	
SL10	81	4♀	♀75–82	♀79	♀3.64	3.29
		3♂	♂74–81	♂78	♂3.43	
SL11	-	3♀	♀16–23	♀19	♀3.75	3.10
		2♂	♂22–23	♂22	♂0.99	
LTS	176	3♀	♀105–160	♀128	♀29.79	29.94
		2♂	♂124–176	♂150	♂36.39	
%LTS/TL	29%	3♀	♀19%–27%	♀22%	♀4.13	4.17
		2♂	♂22%–29%	♂26%	♂4.49	

the examined specimens (Figures 2B, 4D). Only one of the examined females with paradorsal seta on the right side, the remaining four showed the paradorsal seta on the left side. Two pairs of sensory spots, one subdorsal and one laterodorsal, both located behind and close to the muscular scars (Figures 2B, 4B). One pair of laterodorsal longitudinal ridges reaching the anterior edge of the segment. With blunt anterior middorsal projection, only visible with DIC, never protruding beyond posterior margin of segment 1 (Figures 2B, 4D). Sternal plates with a pair of ventromedial groove-like, curved muscular scars and with a pair of ventromedial sensory spots. Reticulate pattern of cuticular wrinkles from the lateral margins just to the ventromedial longitudinal ridges (Figure 4C). Females furthermore with a pair of ventrolateral setae (Figures 2C, 4C). Males always with one pair of large, ventromedial tubes ventrome-

Table III. Summary of location of setae and sensory spots in *Pycnophyes almansae* sp. nov. arranged by series. Abbreviations: LD, laterodorsal; LV, lateroventral; PD, paradorsal; PL, paralateral; SD, subdorsal; VL, ventrolateral; VM, ventromedial; f, female condition of sexually dimorphic character; m, male condition of sexually dimorphic character; lts, laterotermial spines; ps, penile spines; se, setae; ss, sensory spots; t, tubes; 1, only one unpaired cuticular structure; 2, two pairs of cuticular structures.

Segment	PD	SD	LD	PL	LV	VL	VM
1		ss	ss	se		se	ss
2	se(1),ss	ss	se,ss		se	se(f)	ss,t(m)
3	se,ss	ss	se,ss		se		se,ss
4	se,ss	ss	se		se		se,ss
5	se,ss	ss	se,ss			se	se,ss
6	se,ss	ss	se		se		se,ss
7	se,ss	ss	se,ss		se		se,ss
8	se,ss	ss,ss	se		se		se,ss
9	se,ss	ss,ss	ss		se(m)		se,ss
10		ss	ss		se	se	ss
11					lts	ps(2,m)	

dially on the sternal plates and without ventrolateral setae. Conspicuous longitudinal band of cuticular hairs along the tergosternal junctions. Dorsal secondary fringe near the anterior margin, parallel with the segment edge. Three parallel ventral secondary fringes, the anterior-most and posterior-most extend from the lateral margins to a ventromedial position. The median secondary fringe is limited to the ventrolateral margin. Anterior and median secondary fringes with a lateral indentation (Figure 2A,B).

Segment 3. Tergal plate with a pair of paradorsal setae flanking a hairy middorsal elevation with associated paradorsal atria and sensory spots similar to those on segment 2 (Figures 3D,G, 4D). Two additional pairs of setae, one laterodorsal and one lateroventral. Two pairs of sensory spots, one subdorsal behind a pair of muscular scars, similar to those of segment 2; the other one laterodorsal, located more laterally than the laterodorsal setae (Figures 2B, 4B). Sternal plates with a pair of ventromedial setae and slightly more lateral sensory spots (Figures 2A, 4A,C). One pair of muscular scars similar to those on segment 2. Hairy tergosternal junctions, pectinate fringe, secondary fringes, anterior longitudinal ridges and reticulate pattern of cuticular wrinkles same as those on segment 2.

Segment 4. Tergal and sternal plates with the same cuticular features as those on segment 3, except for the absence of laterodorsal sensory spots and the presence of 2 4 long hairs located over the middorsal elevation, mostly recognisable with SEM (Figures 2A,B, 4B,D).

Segment 5. Cuticular features of tergal plate same as on segment 3, except for the absence of lateroventral setae. Sternal plates same as on segment 3, but with a pair of ventrolateral setae (Figures 2A, 3E, 4A,B).

Segment 6. Tergal and sternal plates with the same cuticular features as those on segment 4 (Figures 4A,B,E).

Segment 7. Tergal and sternal plates same as those on segment 3, but with 2 4 long hairs on the middorsal elevation, mostly recognisable with SEM (Figure 3F, 4A,B,E,F).

Segment 8. Tergal and sternal plates same as those on segment 4, except for the presence of an additional pair of subdorsal sensory spots (Figure 4A,B,E,F). Variations in the position of the ventromedial setae have been observed in some specimens. These variations were displayed as a displacement of these setae towards the midventral line, although never reaching the paraventral position.

Segment 9. Tergal plate similar to the one on segment 8, except for the absence of laterodorsal setae; one pair of sensory spots appears at this position instead (Figure 4B). Middorsal elevation with 2 4 long hairs. Females without lateroventral setae. Cuticular features of sternal plates same as those on segment 3. Ventromedial setae displaced towards the midventral line, although never reaching the paraventral position (Figure 4A,F). Some specimens showed these setae not displaced, in the same position as in segment 7. One pair of prominent apodemes or anteromesial thickenings of ventral pachycycli (Mittelwülste) near the anterior margin of segment (Figures 2A,B, 4B). These apodemes may be lacking in specimens, independent of the sex.

Segment 10. Tergal plate with one pair of lateroventral setae and one pair of subdorsal and laterodorsal sensory spots, the latter near the posterior margin of the segment (Figures 2B, 4B). Middorsal elevation, setae or subcuticular atria not present. Sternal plates with muscular scars, similar to those on the previous segments. One pair of ventrolateral setae and one pair of ventromedial sensory spots (Figure 3H). Anteromesial apodemes or thickenings of ventral pachycycli (Mittelwülste) present near anterior margin (Figure 4A,B). A single secondary fringe appears, equivalent to the anteriormost one of preceding segments. Other cuticular features same as those on previous segments.

Segment 11. With lateral terminal spines. Posterior margin of tergal plate shows short hairs (Figure 4G). Males with two pairs of flexible penile spines of equal size and genital pores surrounded by a tuft of long hairs (Figures 2A,D, 4H).

Etymology

The species name is dedicated to Ms. Hilaria Almansa, grandmother of the first author, who lived for many years near the type locality.

Sexual dimorphism

Males always with a pair of large tubes in ventromedial position on segment 2 and females with a pair of ventrolateral setae. Females without lateroventral setae on segment 9. Males with two pairs of penile

spines and a tuft of long hairs surrounding the genital pore.

Associated kinorhynch fauna

Antygomonas sp. 1, *Echinoderes cantabricus*, *E. dujardinii*, *E. hispanicus*, *Echinoderes* sp. 1, *Echinoderes* sp. 2, *Pycnophyes aulacodes*, *P. carinatus* Zelinka, 1928, *P. communis* Zelinka, 1908, *P. dentatus* (Reinhard, 1881), *P. dolichurus*, and *P. zelinkaei* Southern, 1914.

Many species of *Pycnophyes* present middorsal elevations and paradorsal subcuticular atria on segments 2 9 as does *P. almansae* sp. nov. However, only nine species possess paradorsal or middorsal setae on all segments from 2 to 9. These include *P. australensis* Lemburg, 2002, *P. carinatus*, *P. corrugatus* Higgins, 1983, *P. dentatus*, *P. faveolus* Brown, 1985, *P. flaveolatus* Zelinka, 1928, *P. newguiniensis* Adrianov, 1999 (see Adrianov & Malakhov 1999), *P. newzealandensis* Adrianov, 1999 (see Adrianov & Malakhov 1999) and *P. parasanjuanensis* Adrianov & Higgins, 1996. Nevertheless, *P. australensis*, *P. corrugatus*, *P. dentatus* and *P. flaveolatus* show a single seta in the middorsal position on segments 2 9 and they never have a pair of paradorsal setae on any of these segments (Zelinka 1928; Higgins 1983; Lemburg 2002). Moreover, *P. dentatus* has a conspicuous and wide net-like structure in the anterior area of segment 1 (Reinhard 1881; Zelinka 1928; pers. obs.) that appears inconspicuous and very narrow in the new species.

Remarks

Pycnophyes carinatus, *P. faveolus*, *P. newguiniensis*, *P. newzealandensis* and *P. parasanjuanensis* present middorsal or paradorsal setae on all segments 2 9 and one pair of paradorsal setae on at least one of these segments (Zelinka 1928; Brown 1985; Adrianov & Higgins 1996; Adrianov & Malakhov 1999). Among these species, *P. parasanjuanensis* is the only one with a pair of setae flanking the middorsal elevation, only on segment 6, whereas *P. almansae* sp. nov. has a pair of paradorsal setae on segments 3 9 and a single paradorsal seta on segment 2. Moreover, the net-like decoration in the anterior-most region of the first trunk segment is clearly much more developed in *P. parasanjuanensis* than in the new species (Adrianov & Higgins 1996). On the other hand, *P. carinatus* and *P. newzealandensis* differ from *P. almansae* sp. nov. by the presence of paradorsal setae on segment 1, which is absent in the new species. In addition, these latter two species have a pair of paradorsal setae on segment 2, whereas *P. almansae* sp. nov. has a single paradorsal seta on this segment (Zelinka 1928; Adrianov & Malakhov 1999). *P. newzealandensis* also differs from *P. almansae* sp. nov. by the absence of ventrolateral setae on segment 5 (Adrianov & Malakhov 1999).

Pycnophyes newguiniensis and *P. almansae* sp. nov. share the presence of ventrolateral setae on segment 5 and paradorsal setae on all segments 2 9, but *P. newguiniensis* has a single middorsal seta on segment 1, which is lacking in the new species. Moreover, the paradorsal seta on segment 2 is single in *P. almansae* sp. nov. but paired in *P. newguiniensis* (Adrianov & Malakhov 1999). Finally, although both *P. faveolus* and *P. almansae* sp. nov. have pairs of paradorsal setae on segments 3, 5 and 7 9, the former species has only a single seta on segments 1 2, 4 and 6. Moreover, *P. faveolus* differs from the new species by the lack of ventrolateral setae on segment 5 (Brown 1985).

Characters such as the distribution of sensory spots are not very reliable for comparative purposes, bearing in mind that the classical descriptions of many species does not always draw attention to them or with enough detail; for example, the original description of *P. newguiniensis* does not include any information about them (Adrianov & Malakhov 1999), although these authors do so while describing other species in the same paper. Nevertheless, the distribution of sensory spots in *Pycnophyes almansae* sp. nov. differs from both *P. carinatus*, *P. newzealandensis* and *P. faveolus*: *P. carinatus* has pairs of subdorsal sensory spots on segments 1 2 (both with two pairs), 3 6, 7 9 (two pairs) and 10; laterodorsal ones on segments 1 10; ventrolateral ones on segment 9; and ventromedial ones on segments 1 (with two pairs), 2 10 (Zelinka 1928; pers. obs.); *P. newzealandensis* has pairs of ventromedial sensory spots only, on segments 3 9 (Adrianov & Malakhov 1999); and lastly, *P. faveolus* has pairs of subdorsal sensory spots on segments 1 10; laterodorsal ones on segments 1 10; ventrolateral ones on segments 2 (only in males) and 3 10; and ventromedial ones on segment 1 (Brown 1985).

From the analysis of all these characters, we can conclude that *P. almansae* sp. nov. can easily be distinguished from any other species in the genus.

***Pycnophyes lageria* sp. nov.**

Figures 5 9; Tables IV V

Material examined

A total of 14 specimens (8 males and 6 females) were examined with DIC, and 5 additional specimens (4 males and 1 female) were examined with SEM. The specimens were collected at the type locality, Cádiz Bay, S Spain (see Table I for detailed

sampling locations and coordinates). The type series includes the holotypic adult male (locality: Cádiz Bay, 36°29.798′N, 6°12.871′W, 11 November 2011), allotypic adult female (same date and locality) and 5 paratypes, 3 males and 2 females (locality: Cádiz Bay, 36°31′34.95″N, 06°13′31.75″W, 23 Septmeber 1993). All type specimens are mounted in Fluoromount-G® and deposited at the Zoological Museum, University of Copenhagen under accession numbers ZMUC KIN-621 (holotype), ZMUC KIN-622 (allotype) and ZMUC KIN-623 to KIN-627 (paratypes). Additional specimens are deposited in the Marine Zoology Lab., Dept. of Zoology and Anthropology, Universidad Complutense de Madrid.

Diagnosis

Pycnophyes without conspicuous middorsal structures (neither middorsal processes nor middorsal elevations) or subcuticular atria on any segment. Tergal anterior edge of segment 1 smooth, followed by a characteristic cuticular area resembling candle wax drops with a net-like ornamentation inside. Laterodorsal setae on segments 2–9; paralateral ones on segment 1; lateroventral ones on segments 2, 4–6, 8 and 10 (the latter with two pairs of setae); ventrolateral ones on segment 1; and ventromedial on segments 2 (present in females only) and 3–9. Middorsal and paradorsal setae not present. Type 1 sensory spots with 8–10 papillae similar to *Nanaloricus* flosculi. Characteristic cuticular decoration with lines of

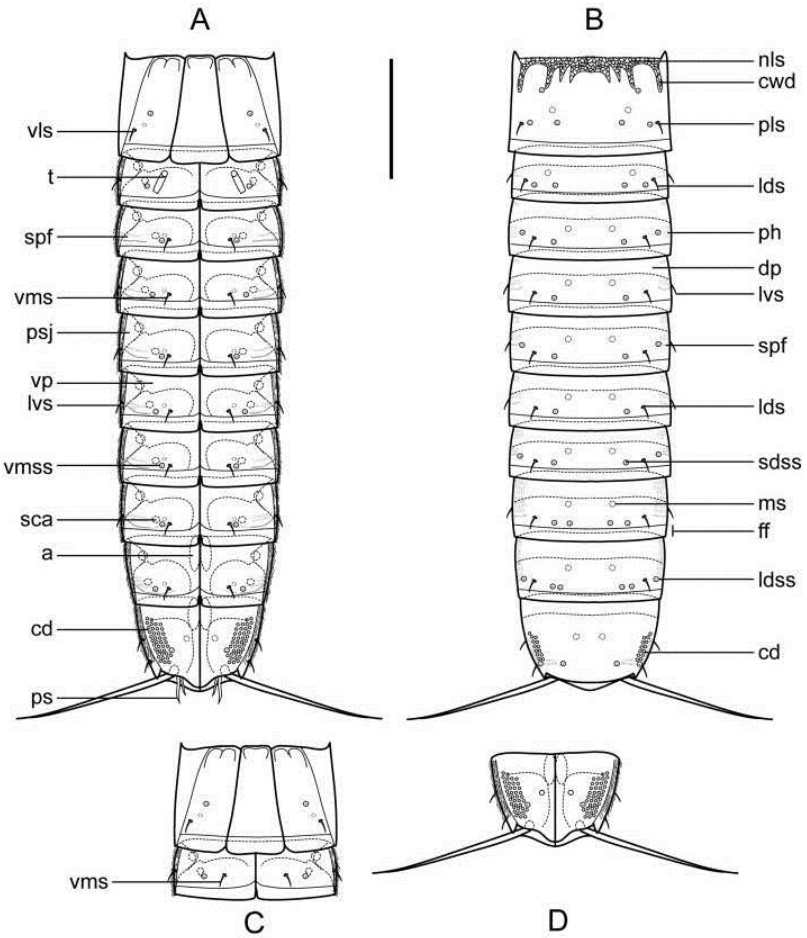


Figure 5. Line art illustrations of *Pycnophyes lageria* sp. nov. (A) Male, ventral view; (B) male, dorsal view; (C) female, segments 1 2, ventral view; (D) female, segments 10 11, ventral view. Scale bar: 100 µm. Abbreviations: a, apodeme, anteromesial thickenings of ventral pachycycli (Mittelwülste); cd, cuticular depressions; cwd, lobulated profile as candle wax drops; dp, dorsal pachycycli; ff, free flap; lds, laterodorsal seta; ldss, laterodorsal sensory spot; lvs, lateroventral seta; ms, muscular scar; nls, net-like structure; ph, patch of hairs; pls, paralateral seta; ps, penile spine; psj, peg and socket joint; sca, subcuticular atria of sensory spot; sdss, subdorsal sensory spot; spf, secondary pectinate fringe; t, tube; vls, ventrolateral seta; vms, ventromedial seta; vmss, ventromedial sensory spot; vp, ventral pachycycli.

Table IV. *Pycnophyes lageria* sp. nov. Measurements (μm) for holotype and means of studied specimens, females and males. Abbreviations: LTS, laterotermínal spine; MSW-5, maximum sternal width (on segment 5); *n*, number of measured specimens; SD, standard deviation; SL, segment length; SW-10, standard width (on segment 10); TL, total length; ♀, female; ♂, male.

Character	Holotype	*n*	Range	Mean	SD	SD ♀–♂
TL	534	3♀	♀507–564	♀534	♀28.32	22.61
		5♂	♂487–534	♂515	♂17.92	
MSW-5	145	1♂	–	–	–	–
SW-10	113	1♂	–	–	–	–
SL1	83	3♀	♀79–85	♀82	♀3.24	3.07
		5♂	♂76–83	♂79	♂2.85	
SL2	47	3♀	♀47–52	♀50	♀2.87	2.92
		5♂	♂44–48	♂46	♂1.63	
SL3	51	3♀	♀49–51	♀50	♀0.99	3.23
		5♂	♂42–51	♂47	♂3.71	
SL4	55	3♀	♀52–57	♀54	♀2.68	3.37
		5♂	♂47–55	♂51	♂3.25	
SL5	55	3♀	♀58–59	♀59	♀0.60	3.75
		5♂	♂50–55	♂52	♂2.17	
SL6	55	3♀	♀60–60	♀60	♀0.17	4.41
		5♂	♂49–56	♂53	♂2.78	
SL7	57	3♀	♀58–60	♀59	♀0.79	3.59
		5♂	♂51–57	♂53	♂2.48	
SL8	60	3♀	♀58–60	♀59	♀1.03	3.50
		5♂	♂51–60	♂55	♂3.25	
SL9	58	3♀	♀58–63	♀59	♀2.77	4.20
		5♂	♂52–58	♂55	♂2.11	
SL10	76	3♀	♀69–75	♀73	♀3.58	4.85
		5♂	♂63–76	♂69	♂5.17	
SL11	-	2♀	♀37–40	♀39	♀2.08	4.87
		3♂	♂32–45	♂38	♂6.72	
LTS	135	3♀	♀96–111	♀101	♀8.15	16.08
		5♂	♂101–136	♂123	♂19.99	
%LTS/TL	25%	3♀	♀18%–20%	♀19%	♀0.62	3.03
		5♂	♂21%–26%	♂24%	♂2.11	

circlets (DIC) or round, depressed cuticular areas (SEM) on the ventral region of segment 10 that extend laterally to the dorsal plate. See Figures 5, 8 and 9.

Description

Dimensions and measurements of the examined specimens are summarized in Table IV. The arrangement and distribution of cuticular trunk structures are summarized by segment in Table V.

Mouth cone cylindrical and smooth, with no special cuticular features. Nine oral styles, thin, long and flexible, of similar sizes, located as one anterior to each introvert sector, except in the middorsal sector 6, where a style is absent. Styles consist of only one piece, without articulation sites (Figure 6A,C,D).

Table V. Summary of location of setae and sensory spots in *Pycnophyes lageria* sp. nov. arranged by series. Abbreviations: LD, laterodorsal; LV, lateroventral; PL, paralateral; SD, subdorsal; VL, ventrolateral; VM, ventromedial; f, female condition of sexually dimorphic character; m, male condition of sexually dimorphic character; lts, laterotermínal spines; ps, penile spines; se, setae; ss, sensory spots; t, tubes; tss, twin of sensory spots; 2, two pairs of cuticular structures.

Segment	SD	LD	PL	LV	VL	VM
1	ss	ss,ss	se		se	ss
2	ss	se,ss		se		ss,se(f),t(m)
3	ss	se,ss				se,ss
4	ss	se		se		se,ss
5	ss	se,ss		se		se,ss
6	ss	se		se		se,ss
7	ss	se,ss				se,ss
8	ss,ss	se		se		se,ss
9	tss	se,ss				se,ss
10	ss	ss		se,se		ss
11				lts	ps(2,m)	

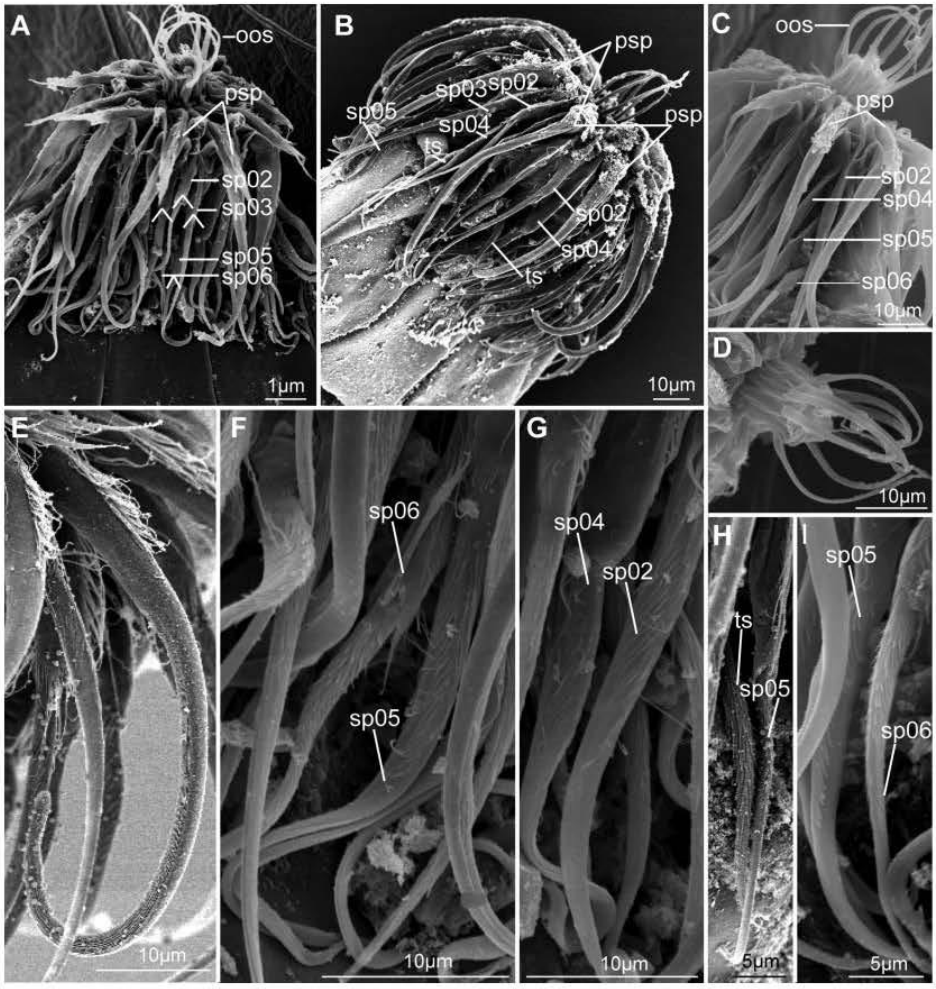
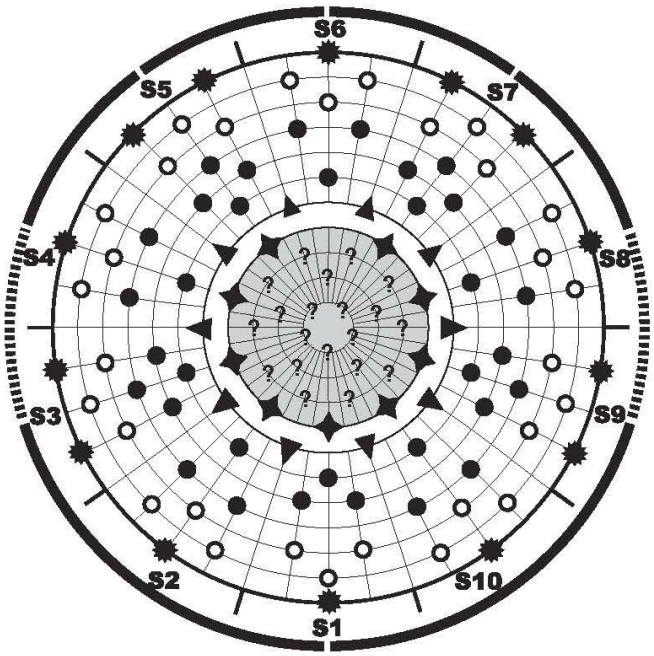


Figure 6. Introvert of *Pycnophyes lageria* sp. nov., SEM photographs. (A) Sector 1, ventral view; (B) sectors 2 and 3, lateral view; (C) sector 4, lateral view; (D) outer oral styles, lateral view; (E) sector 7, spinoscalid; (F) sector 2, fringed scalids; (G) sector 10, smooth scalids; (H) sector 8; (I) sector 4, fringed scalids. Abbreviations: oos, outer oral style; psp, primary spinoscalid; sp, spinoscalid; ts, trichoscalid. Digits after the labels refer to the introvert ring numbers. Lambda symbols (Λ) mark attachment points of scalids.

The introvert has six rings of scalids and one additional ring of trichoscalids. Scalids of the successive rings decrease in general size posteriorly. When the introvert is fully extended, a wide nude area of flexible cuticle appears between the neck placids and the scalids. Ring 01 has 10 primary spinoscalids consisting of two articulated pieces, one short proximal piece and one long, distal endpiece. The proximal piece is equipped with a fringe of long hairs located on the upper edge only and absent on the lateral margins. The terminal third of the proximal piece is free, pointing outwards and overlapping the posteriorly directed endpiece (Figure 6A,C). The endpiece has a fringe of long hairs only near the base, whereas the remaining part has a smooth dorsal side and a striated ventral side (Figure 6E). All primary spinoscalids terminate in a blunt tip (Figure 6E). Smooth spinoscalids are present in rings 02–04. Ring 02 is formed by 10 spinoscalids alternating in position with the primary spinoscalids of ring 01, all with a basal sheath and a long endpiece (Figure 6A–C). The basal sheath of each spinoscalid appears hairy along the upper edge and terminates into two lateral tufts. The endpiece has lateral hairs proximally, and appears bald distally (Figure 6G). Ring 03 with 10 spinoscalids, resembling those of ring 02 but with a wider triangular cross-section and a longer hairy area on the lateral regions of the endpiece (Figure 6A–C). Ring 04 formed by 14 spinoscalids, similar to those of ring 03 (Figure 6G). Fringed spinoscalids in rings 05–06. Rings 05 and 06 with 15 spinoscalids each, all similar to those on previous



Scalid and style arrangement

Ring/Sector		1	2	3	4	5	6	7	8	9	10	Total
00 oos	✦	1	1	1	1	1	0	1	1	1	1	9
01	▼	1	1	1	1	1	1	1	1	1	1	10
02	●	1	1	1	1	1	1	1	1	1	1	10
03	●	2	0	2	0	2	0	2	0	2	0	10
04	●	0	2	1	2	1	2	1	2	1	2	14
05	○	2	1	2	1	2	1	2	1	2	1	15
06	○	1	2	1	2	1	2	1	2	1	2	15
07 Tr	✸	1	1	2	1	2	1	2	1	2	1	14
Total scalids		7	7	9	7	9	7	9	7	9	7	88

Figure 7. Diagram of mouth cone (grey area), introvert and placids in *Pycnophyes lageria* sp. nov. with the distribution and type of scalids by ring and sector. Dashed lines mark flexible cuticular lateral areas. 'Double diamonds' are marked in the table with continuous lines. oos, outer oral styles; Tr, trichoscalid.

rings but more rounded in cross-section and a dense fringe of short hairs on their upper proximal edge (Figure 6F,H,I). The seventh ring has 14 trichoscalids directly attached on the introvert, without intermediary trichoscalid plates. All trichoscalids pointed and uniformly covered by hairs (Figure 6H).

The introvert can also be described by sectors: every two consecutive primary spinoscalids can be considered as delimiting a sector in the introvert (Figure 6A C), which is consequently divided into 10 sectors, numbered clockwise from the midventral one. Even sectors contain 6 spinoscalids (Figure 6B,C), whereas odd sectors have 7 (Figure 6B), except sector 1 which has 6 (Figure 6A). Only odd sectors present spinoscalids in the ring 03 and show a 'double diamond' arrangement of their 7 scalids. Even sectors show a scalid quincunx formed by the rings 04 06. See Figure 7 for a complete summary of oral styles and scalid numbers and arrangement.

Neck. With 4 dorsal and 2 ventral placids. All placids are apparently robust, hard, with a concave surface. Dorsal placids are of similar size and nearly square profile. Ventral placids widely rectangular, extending from the lateral margin of the episternal plate to the midventral line. All placids are joined to the anterior edge of segment 1 by one of their long sides and continue anteriorly with the thin and flexible cuticle of the introvert.

Trunk. With 11 segments. First segment with one tergal plate and three sternal plates. Segments 2 11 with one tergal plate and two sternal plates (Figures

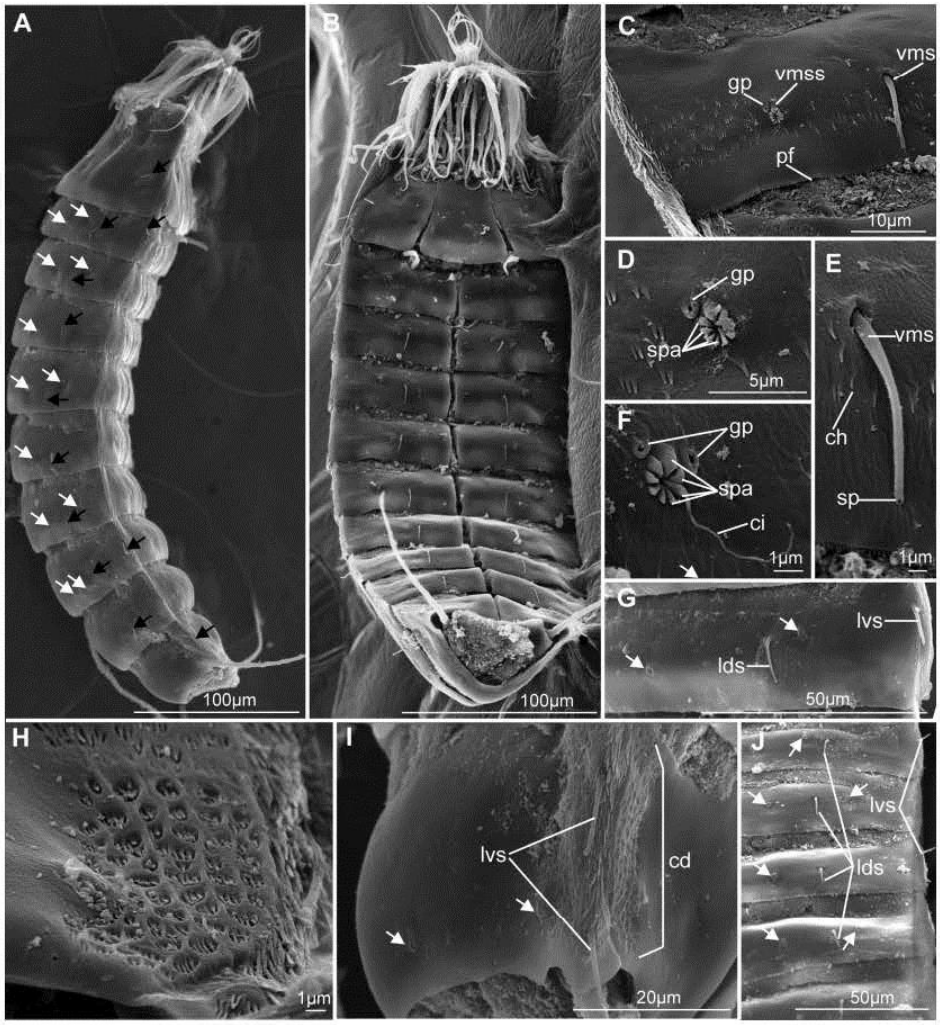


Figure 8. *Pycnophyes lageria* sp. nov., SEM photographs. (A) Male, lateral view; (B) male, ventral view; (C) left sternal plate of segment 8; (D) detail of segment 8 showing sensory spot formed as N-flosculum; (E) detail of segment 8 showing ventromedial seta; (F) detail of segment 7 showing sensory spot formed as N-flosculum; (G) female with modified position of sensory spots on segment 2, laterodorsal view; (H) male, segment 10, ventral view; (I) male, segment 10, lateral view; (J) segments 2–5, laterodorsal view. Abbreviations: cd, cuticular depressions; ch, cuticular hair; ci, cilium; gp, gland pore; lds, laterodorsal seta; lvs, lateroventral seta; pf, pectinate fringe; sp, seta pore; spa, sensory papillae; vms, ventromedial seta; vmss, ventromedial sensory spot. Sensory spots are marked with white arrows. Setae are marked with black arrows.

5, 8A,B, 9A,B). Cuticle relatively thin, with one pair of ventromedial longitudinal ridges and with well-developed, thick pachycycli throughout the whole trunk. Conspicuous, equally sized peg and socket joints (tergosternal articulations) from segments 2 to 9 (Figures 5A, 9A). The segment width is fairly constant between segments 2 and 7, with a conventional maximum width at segment 5. Middorsal structures and subcuticular atria absent on all segments. One pair of conspicuous muscular scars in subdorsal and ventromedial positions present on segments 1–10 (Figures 5, 9A,B,F). Posterior margin of tergal and sternal plates (free flap) smooth, overlapping the anterior edge of the following segment (Figures 5A,B, 9A,B,F). Minute pectinate fringe, only perceptible with SEM.

Segment 1. Lateral margins of tergal plate project anteriorly into horn-like extensions. Tergal anterior edge of segment smooth, not denticulated; anterior margin formed by a characteristic cuticular area,

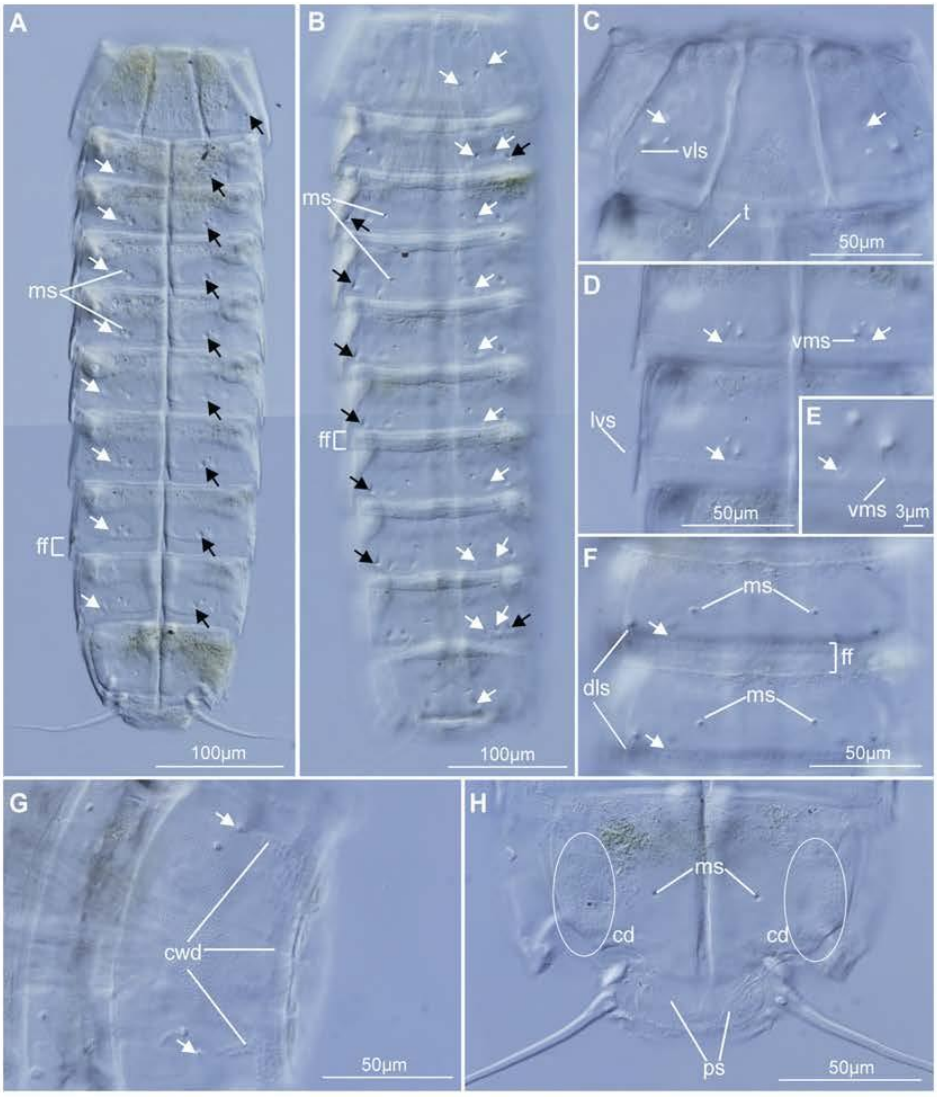


Figure 9. *Pycnophyes lageria* sp. nov., DIC photographs. (A) Female, ventral view; (B) female, dorsal view; (C) male, segments 1 2, ventral view; (D) segments 7 8, ventral view; (E) detail of segment 7 showing ventromedial seta; (F) segments 7 8, dorsal view; (G) female, segment 1, dorsal view; (H) male, segments 10 11. Abbreviations: cd, cuticular depressions; cwd, lobulated profile as candle wax drops; dls, dorsolateral seta; ff, free flap; lvs, lateroventral seta; ms, muscular scar; ps, penile spine; t, tube; vls, ventrolateral seta; vms, ventromedial seta. Sensory spots are marked with white arrows. Setae are marked with black arrows.

with a lobulated profile resembling candle wax drops and net-like ornamentation (Figures 5B, 9G). Tergal plate with a pair of paralateral setae and three pairs of sensory spots, two of them laterodorsal and one subdorsal (Figures 5B, 8A, 9B). Sensory spots on this and all the following segments with a collar of 8–10 papillae or petals surrounding a central pore through which a cilium emerges. Sensory spots usually with one or two adjacent drop-shaped pores outside the petal crown (Figure 8C,D,F). Ventral side with one trapezoid midsternal plate flanked by two episternal plates, all with round corners. Each episternal plate with a ventromedial sensory spot and muscular attachment, both located in the middle region of the plate, and a ventrolateral seta at the posterior third of the segment (Figures 5A,C, 9C). Pectinate fringe inconspicuous in the dorsal region but slightly developed ventrally, only detectable with SEM.

Segment 2. Tergal plate with one pair of lateroventral and laterodorsal setae. Two pairs of sensory spots present: one laterodorsal, close mesially to the laterodorsal seta; and one subdorsal (Figures 5B, 8A,J, 9B). Females sometimes with the laterodorsal sensory spots located at the opposite outer side of the laterodorsal seta (Figure 8G). Sternal plates with a pair of ventromedial sensory spots. Females also with a pair of ventromedial setae (Figure 5C). Males always with one pair of large tubes located ventromedially on the sternal plates near the anterior margin (Figures 5A, 9C). Conspicuous, longitudinal band of cuticular hairs along the tergosternal junctions (Figure 5A). Secondary fringe reduced to a short row of cuticular hairs, only present on the lateral regions of tergal plate.

Segment 3. Tergal plate with one pair of laterodorsal setae, and one pair of sensory spots, laterodorsally and subdorsally (Figure 8A,J). Without lateroventral setae. Sternal plates with a pair of ventromedial setae mesially adjacent to a sensory spot (Figures 5, 8A,B, 9A). Secondary fringe on dorsal side reduced to patches of cuticular hairs on the lateral regions of tergal plate. Secondary fringes on ventral side as two parallel rows of cuticular hairs located close to the ij-line, and running from the lateral to the ventromedial regions (Figure 5). Hairy tergosternal junctions as on segment 2.

Segment 4. Tergal plate with one pair of lateroventral and laterodorsal setae. One pair of subdorsal sensory spots (Figures 5B, 8A,J, 9B). Sternal plates similar as those on segment 3, except for the ventromedial sensory spots clearly separated from the ventromedial seta (Figures 5A, 9A). Secondary fringe on dorsal side limited to the lateral regions as one row and an anterior patch of cuticular hairs. Hairy tergosternal junctions and secondary fringes on ventral side same as on the preceding segment.

Segment 5. Tergal and sternal plates same as on segment 3, but with a pair of lateroventral setae. Laterodorsal patches of cuticular hairs slightly wider than on the previous segment (Figures 5A,B, 8A,J).

Segment 6. Tergal and sternal plates same as on segment 4, but with the hairy patches on the tergal plate same as those on segment 5 and with scattered additional cuticular hairs between both secondary fringes on the ventral side (Figures 5A,B, 8B).

Segment 7. Tergal and sternal plates same as those on segment 3, except for wider hairy patches on the tergal plate (Figures 8A,B,F, 9D F).

Segment 8. Tergal plate similar to segment 4, but with additional pair of subdorsal sensory spots (Figures 8A,C E, 9F). Sternal plates same as those on segment 3 (Figure 9D). Secondary fringes on the tergal plate extend to the laterodorsal setae, and hairy patches appear as broad longitudinal bands along the laterodorsal region of the segment (Figures 5B, 8C). Secondary fringes on the sternal plates with an additional third row of cuticular hairs, and with a couple of hairs extending to the ventromedial setae (Figure 5A). Hairy tergosternal junctions same as previous segments (Figure 8C).

Segment 9. Tergal plate without lateroventral setae, but with a pair of laterodorsal setae. Three pairs of sensory spots, one of them laterodorsal and two very close subdorsal, making it twin sensory spots (Figures 5B, 8A, 9B). Sternal plates the same as those on segment 4. One pair of apodemes or anteromesial thickenings of ventral pachycycli (Mittelwülste) near anterior margin of segment. Hairy tergosternal junctions same as on previous segments. Secondary fringes on the dorsal side similar to those on segment 8. Secondary fringe on the ventral side as a single row extending to the ventromedial position and patches of cuticular hairs on the lateral regions (Figure 5A,B).

Segment 10. Tergal plate with two pairs of lateroventral setae, medially and posteriorly, and a pair of laterodorsal and subdorsal sensory spots near the posterior margin of the segment (Figures 5B,D, 8I). Ventral region showing several longitudinal lines of cuticular annuli visible with DIC (Figures 5B,D, 9H). SEM images reveal such structures as round cuticular depressions with two or three small hairs inside (Figure 8H). These cuticular structures extend from the lateral margins of tergal plate to the laterodorsal sensory spots (Figures 5A,B, 9G). With a pair of ventromedial sensory spots adjacent to the cuticular annuli area (Figure 9J). Ventral margin of sternal plates rounded and protruding posteriorly in the midventral region (Figures 5A, 9H). Anteromesial thickenings of ventral pachycycli (apodeme, Mittelwülste) present near anterior margin (Figure 9H). Tergosternal junctions hairy as on the previous segments and showing a posterior pointed projection (Figures 5A, 9A,H). Secondary fringe only present along the laterodorsal regions, between the sensory spots.

Segment 11. With lateral terminal spines. Males with two pairs of flexible penile spines (Figures 5A,D, 9H).

Etymology

The species name, *lageria*, refers to La Geria, a region in Lanzarote (Canary Islands) where vineyards are arranged into fields of holes (geria or lageria) resembling the cuticular sculpturing of segment 10 in the new species.

Sexual dimorphism

Males always with a pair of large tubes in ventromedial position on segment 2 and females with a pair of ventromedial setae. Some females with the laterodorsal sensory spots on segment 2 located at the outer side of the laterodorsal setae. Males with two pairs of penile spines.

Associated kinorhynch fauna

Echinoderes cantabricus, *E. dujardinii*, *E. hispanicus*, and *E. worthingi* Southern, 1914; *Pycnophyes communis* and *P. dentatus*.

Remarks

Diagnostic characters of *Pycnophyes lageria* sp. nov., such as the absence of both middorsal processes or middorsal elevations and subcuticular atria in all segments, are shared with five species in the genus: *P. corrugatus*, *P. ecphantor* Higgins, 1983, *P. egyptensis* Higgins, 1966, *P. faveolus* and *P. sculptus* Lang, 1949. The first four species differ from *P. lageria* sp. nov. by the presence of middorsal setae on some segments: *P. corrugatus* on segments 2 9 (Higgins 1983), *P. ecphantor* on segments 2, 4 8 (Higgins 1983), *P. egyptensis* on segments 2 8 (Higgins 1966) and *P. faveolus* on segments 1 2, 4 and 6 (Brown 1985). The latter also has pairs of paradorsal setae on segments 3, 5 and 7 9. Such characters are absent in the new species. Only *P. sculptus* shares the complete lack of middorsal structures with *P. lageria* sp. nov. No middorsal or paradorsal setae, processes, elevations or subcuticular atria are present (Lang 1949). Despite this, *P. lageria* sp. nov. shows several features that clearly differentiate both species: the characteristic tergal anterior margin of segment 1 with a lobulated profile resembling candle wax drops and net-like decoration, the longitudinal lines of cuticular annuli with cuticular hairs inside on the ventral region of segment 10 that extend to the dorsal plate, the absence of both ventromedial and laterodorsal setae on segment 10 and different distribution of lateroventral setae; hence, the species described herein is easily distinguished from *P. sculptus*.

On the other hand, *P. lageria* sp. nov. shows a characteristic ornamentation on the tergal anterior margin of segment 1 and on the ventral and lateral regions of segment 10. Some species such as *P. dentatus*, *P. faveolus*, *P. furugelmi* Adrianov, 1999, *P. iniorhaptus* Higgins, 1983, *P. parasanjuanensis* and *P. sanjuanensis* Higgins, 1961 have some kind of ornamentation on the tergal anterior margin of segment 1, but none of them has a lobulated profile resembling candle wax drops. Regarding the special ornamentation on segment 10, three species have some cuticular decoration: *P. corrugatus*, *P. dentatus* and *P. iniorhaptus*. The first two species have vertical cuticular striations near lateral margins, whereas *P. iniorhaptus* has punctuated cuticular sculpturing near lateral margins on segments 2 10. These cuticular markings are clearly different from the little circular depressions described herein for the new species. Subsequently, *Pycnophyes lageria* sp. nov. has unique characters that are not present in any other species in the genus.

The special nature of the sensory spots of this new species deserves some comments. *P. lageria* sp. nov. possesses sensory spots with a reduced number (8 10) of cuticular papillae. These types of sensory spots have been reported from only three other kinorhynch species, none of them belonging to the genus *Pycnophyes*: the cyclorhagid *Echinoderes intermedius* Sørensen, 2006 and the homalorhagids *Kinorhynchus yushini* Adrianov, 1989 (see Adrianov & Malakhov 1994) and *Paracentrophyes anurus* Sørensen et al., 2010 (Sørensen et al. 2010). Moreover, the sensory spots of *P. lageria* sp. nov. have one or two associated external pore glands, a feature that has not been described for those three species. The association between sensory spots and gland cells is quite common in kinorhynchs (Adrianov et al. 1989; Adrianov & Malakhov 1990; Kristensen & Higgins 1991; Nebelsick 1992; Adrianov & Malakhov 1994; Neuhaus & Higgins 2002); however, the gland pore is always located within the sensory spot and not outside as in *P. lageria* sp. nov.

Although this type of sensory spot is unusual in adult kinorhynchs, it is quite common in juvenile stages of at least the genera *Paracentrophyes* (see Neuhaus 1995), *Pycnophyes* (see Neuhaus 1993) and *Kinorhynchus* (see Adrianov et al. 1989; Kristensen & Higgins 1991; Adrianov & Malakhov 1994). This cuticular specialization resembles the flosculi of Priapulida (*Tubiluchus)* (Van der Land 1970; Kirsteuer 1976) and Loricifera (*Nanaloricus* flosculi, N-flosculi) (Kristensen 1983; see also Kristensen & Higgins 1991; Nebelsick 1992; Adrianov & Malakhov 1994; Sørensen 2006). Such structures differ

clearly from most other usual kinorhynch sensory spots, which have a high number of papillae (up to 100) arranged randomly in a round to oval depressed area (Kristensen & Higgins 1991; Nebelsick 1992). It is tempting to consider this kind of sensory spot, or N-flosculi, as a plesiomorphic character for kinorhynchs, shared by the most recent common ancestor of kinorhynchs, priapulids and loriciferans.

Introvert. A quite common pattern of scalid distribution in kinorhynchs, when arranged by sectors, is the presence of 'double diamonds' in odd sectors and either quincunxes or 'single diamonds' in the even ones. However, it should be noted that most of the available introvert data are from cyclorhagid species. Very little is known about the morphology and arrangement of introvert structures in species of *Pycnophyes*. Zelinka (1928) described with light microscopy the introvert of a J6 juvenile specimen of *Pycnophyes communis* Zelinka, 1908 a description later assumed to be valid for the adult also by Neuhaus (1995). The only introvert descriptions from *Pycnophyes* species, based on SEM information, are collected in the unpublished master thesis of O. Ziemer (2001): *P. canadensis* Higgins & Korczinski, 1989; *P. cryopygus* Higgins & Kristensen, 1988; *P. chukchiensis* Higgins, 1991; and *P. grenlandicus* Higgins & Kristensen, 1988. All of them share the presence of 'double diamonds' in odd sectors and the number of trichoscalids with *P. lageria* sp. nov. However, the arrangement in even sectors, the same without quincunxes in the four referred species, is different from the new species.

The only published SEM description from a species of Pycnophyidae is *Kinorhynchus phyllotropis* Brown & Higgins, 1983 (see Brown 1989). Remarkably, this species and *P. lageria* sp. nov. share the lack of spinoscalids in ring 03 of the even sectors, the same kind of scalid morphology, the same number of spinoscalids in the odd and even sectors and the same number of trichoscalids. However, *K. phyllotropis* also shows some differences regarding introvert sector 1 and the distribution of trichoscalids by sectors. Nevertheless, this last difference could be just a misinterpretation of the true trichoscalid position a character sometimes difficult to check precisely because of the absence of trichoscalid plates.

The only other homalorhagid introverts described using modern methods so far are those of *P. anurus* (Sørensen et al. 2010) and *P. praedictus* (Higgins 1983; Neuhaus 1995) which belong to the sister family Neocentrophyidae. Regarding the head morphology, the most important difference between these two species and *P. lageria* sp. nov. is the presence of two different types of jointed oral styles, of different sizes (Neuhaus 1995, Sørensen et al. 2010). Nevertheless, the distribution of the scalids in *P. lageria* sp. nov. is very similar to the introvert of the three homalorhagid species referred to above, mostly to *K. phyllotropis*.

Available data on introvert structures mostly include cyclorhagid species. The scalid pattern of the new species (except for introvert sector 1) and the number (14) and arrangement of trichoscalids (7 dorsal and 7 ventral) is shared with three cyclorhagid species: *Antygomonas incomitata* Nebelsick, 1990 (see Sørensen et al. 2009); *Antygomonas oreas* Bauer-Nebelsick, 1996; and *Tubulideres seminoli* Sørensen et al., 2007. However, these species differ from *Pycnophyes lageria* sp. nov. by the structure of the oral styles of the mouth cone.

The new species fits the common pattern of 14 trichoscalids 7 dorsal and 7 ventral found in most kinorhynch genera (Higgins 1990; Neuhaus 1995; Sørensen 2007; Sørensen et al. 2007; Sørensen & Rho 2009; Sørensen & Thormar 2010), a quite stable or conservative arrangement. Only two families, Echinoderidae with 6 trichoscalids and Dracoderidae with 9, differ from this pattern. However, the latter genus shares with *Pycnophyes lageria* sp. nov. the same number and arrangement of dorsal trichoscalids 7, with one in sectors 4, 6 and 8 and two in sectors 5 and 7 apart from the presence of 'double diamonds' in the odd sectors and quincunxes in the even ones (despite the gap in the ring 03) (Sørensen et al. 2012). These data give further support to the proposed close relationship between dracoderids and homalorhagids (Sørensen et al. 2012).

Nevertheless, the available data regarding the introverts are not strong enough to reach any conclusive hypothesis within kinorhynch taxa. A much more comprehensive study on kinorhynch introvert features is now in progress, and it is expected that such a comparative approach will contribute to the still unsolved phylogenetic relationships inside the whole phylum.

Discussion

With the present two new species, the number of *Pycnophyes* species recorded around the Iberian Peninsula reaches nine, with four of them described recently: *Pycnophyes lageria* sp. nov., *P. almansae* sp. nov. (this paper), *P. aulacodes* and *P. dolichurus* (Sánchez et al. 2011). Only one of these species, *P. aulacodes*, has been found outside the Iberian Peninsula, specifically in Tjärnö (Sweden), Hirsholmene (Denmark) and Kaldbak Fjord (Faeroe Islands) (Nuria Sánchez, pers. obs.). New sampling efforts,

both around the Iberian Peninsula and in other European waters, will probably increase the distribution area of these and other homalorhagid species.

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Four new species of *Pycnophyes* (Kinorhyncha: Homalorhagida) from Korea and the East China Sea

Nuria Sánchez, Hyun Soo Rho, Won-Gi Min, Dongsung Kim, Martin Vinther Sørensen

RESUMEN: **Cuatro nuevas especies de *Pycnophyes* (Kinorhyncha: Homalorhagida) de Corea y el mar del este de China.** – Se describen cuatro nuevas especies de aguas coreanas y del este del mar de China. *Pycnophyes pardosi* n. sp. se distingue de las demás especies por la presencia de una única seda paradorsal en los segmentos 2-8 y un par en el segmento 9, combinado con la presencia de engrosamientos cuticulares longitudinales en el lado dorsal y ventral del segmento 10. *Pycnophyes chalgap* n. sp. es fácilmente distinguible por la forma de su placa medioesternal con el margen posterior redondeado, sobrepasando y solapando la mitad anterior del siguiente segmento. *Pycnophyes cristatus* n. sp. es reconocible por la ausencia de sedas ventromediales en los segmentos 3-6 combinado con la presencia de salientes mediodorsales puntiagudos que a partir del segmento 6 se vuelven progresivamente más largos hacia los segmentos posteriores. El saliente del segmento 10 es llamativamente largo, extendiéndose más allá del margen posterior del segmento 11. *Pycnophyes smaug* n. sp. se distingue por su carencia general de sedas que, aparte de las lateroventrales, solo aparecen en posición laterodorsal en los segmentos 2 y 9, y en posición ventromedial en el segmento 9. La descripción de las cuatro especies aumenta hasta 26 el número total de especies válidas de kinorrincos entorno a la península coreana.

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Four new species of *Pycnophyes* (Kinorhyncha: Homalorhagida) from Korea and the East China Sea

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SUMMARY: Four new species of *Pycnophyes* are described from Korean waters and the East China Sea. *Pycnophyes pardosi* n. sp. is distinguished from other congeners by the presence of a single paradorsal seta on segments 2-8 and a pair on segment 9, combined with the presence of longitudinal cuticular thickenings on the dorsal and ventral sides of segment 10. *Pycnophyes chalgap* n. sp. is easily distinguished by the shape of its midsternal plate with the rounded posterior margin, extending beyond and overlapping half of the following segment. *Pycnophyes cristatus* n. sp. is recognized by its lack of ventromedial setae on segments 3-6 combined with the presence of pointed middorsal processes that turn progressively longer towards the posterior segments from segment 6. The process on segment 10 is conspicuously long, extending beyond the posterior margin of segment 11. *Pycnophyes smaug* n. sp. is distinguished by its general scarcity of setae which, with the exception of those in lateroventral positions, only appear in laterodorsal positions on segments 2 and 9 and in ventromedial position on segment 9. The description of four additional species brings the total number of valid kinorhynch species around the Korean Peninsula up to 26.

Keywords: biodiversity, meiofauna, Pycnophyidae, morphology, taxonomy, Korean Peninsula.

RESUMEN: CUATRO NUEVAS ESPECIES DE *PYCNOPHYES* (KINORHYNCHA: HOMALORHAGIDA) DE COREA Y EL MAR DEL ESTE DE CHINA. – Se describen cuatro nuevas especies de aguas coreanas y del este del mar de China. *Pycnophyes pardosi* n. sp. se distingue de las demás especies por la presencia de una única seda paradorsal en los segmentos 2-8 y un par en el segmento 9, combinado con la presencia de engrosamientos cuticulares longitudinales en el lado dorsal y ventral del segmento 10. *Pycnophyes chalgap* n. sp. es fácilmente distinguible por la forma de su placa medioesternal con el margen posterior redondeado, sobrepasando y solapando la mitad anterior del siguiente segmento. *Pycnophyes cristatus* n. sp. es reconocible por la ausencia de sedas ventromediales en los segmentos 3-6 combinado con la presencia de salientes mediodorsales puntiagudos que a partir del segmento 6 se vuelven progresivamente más largos hacia los segmentos posteriores. El saliente del segmento 10 es llamativamente largo, extendiéndose más allá del margen posterior del segmento 11. *Pycnophyes smaug* n. sp. se distingue por su carencia general de sedas que, aparte de las lateroventrales, solo aparecen en posición laterodorsal en los segmentos 2 y 9, y en posición ventromedial en el segmento 9. La descripción de las cuatro especies aumenta hasta 26 el número total de especies válidas de kinorrincos entorno a la península coreana.

Palabras clave: biodiversidad, meiofauna, Pycnophyidae, morfología, taxonomía, península coreana.

INTRODUCTION

Since 2008 a collaborative team anchored in the Korean Institute of Ocean Science and Technology and the Natural History Museum of Denmark has explored the taxonomy and biodiversity of East Asian kinorhynchs. Samples from various localities in the triangle between Malaysia, Micronesia and Korea have been

examined during the study, with the most intensive sampling in the area stretching from the Korean East Sea, through the Korea Strait to the central part of the East China Sea.

The study has already resulted in the description of 13 new species (Sørensen and Rho 2009, Sørensen *et al.* 2010a, 2010b, 2010c, 2012a, 2012b, in press, Lundbye *et al.* 2011) and more descriptions are currently under preparation. The taxonomic work has until now been done with a strong emphasis on cyclorhagid species, and the recently described species include 11 cyclorhagids and only a single homalorhagid, *Paracentrophyes anurus* Sørensen *et al.* 2010. However, the collecting campaigns also revealed several new species of homalorhagid *Pycnophyes* and some of them will be addressed and described in the present contribution. The genus is characterized by species with one tergal and three sternal plates on segment 1, one tergal and two sternal plates on segments 2-11 and a pair of lateral terminal spines on segment 11. Many species bear middorsal processes or elevations along the trunk segments and males usually differ from females by the presence of a pair of ventral tubes on segment 2 and penile spines on segment 11 (Zelinka 1928, Higgins 1983).

So far, only four species of *Pycnophyes* have been recorded from the main study area, i.e. Korea and nearby localities. These include *P. furugelmi* Adrianov, 1999; *P. oshoroensis* Yamasaki *et al.*, 2012; *P. schornikovi* Adrianov, 1999 and *P. tubuliferus* Adrianov, 1989 from the Korean East Sea and Seto Inland Sea (see Adrianov and Malakhov 1999, Murakami *et al.* 2001, Yamasaki *et al.* 2012) and *P. tubuliferus* from Peter the Great Bay and the Korean west coast (Adrianov 1989, Adrianov and Malakhov 1999). Other recordings of homalorhagid kinorhynchs from the area include only *Kinorhynchus yushini* Adrianov, 1989 and *Paracentrophyes anurus* (see Adrianov and Malakhov 1999, Sørensen *et al.* 2010a). One of the reasons for the limited attention paid to homalorhagid taxonomy is may be the difficulties that many researchers experience when trying to identify species of this group. The currently available identification keys are all based on characters that are often highly subjective, ambiguous or only discernible with one particular technique (i.e. only scanning electron microscopy or differential interference contrast).

The ongoing studies have clearly shown that the study area holds a diverse, and so far unrecovered cyclorhagid kinorhynch fauna, and the same pattern appears to exist for homalorhagids. Hence, with the present contribution we can double the number of *Pycnophyes* species known from the area, and contribute even further to the knowledge of kinorhynchs in this diverse region.

MATERIALS AND METHODS

Kinorhynchs were recorded from numerous localities (>35) in the East Sea, Korea Strait and East China Sea during several collecting campaigns organized by the Korean Institute of Ocean Science and Technology between 2006 and 2012. Seven of the sampled stations yielded specimens for the present study (Fig. 1). Station names (MAP-followed by station number) follow the nomenclature used in other recent studies by the authors (see Sørensen *et al.* 2012a, 2012b, in press) to enable a more straightforward comparison of the faunal composition at the localities in past and future publications.

Samples were taken with a Smith-MacIntyre Grab or a box corer and were immediately fixed in formalin.

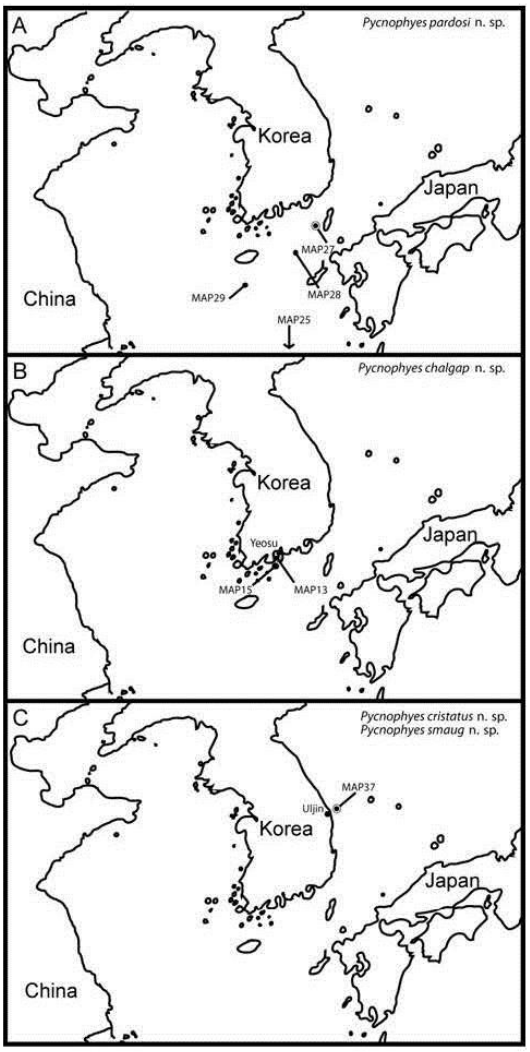

FIG. 1. – Maps showing collecting localities for, *Pycnophyes pardosi* n. sp. (A), *Pycnophyes chalgap* n. sp. (B) and *Pycnophyes cristatus* n. sp. and *Pycnophyes smaug* n. sp. (C). Type localities are marked with a ring around the dot.

Subsequently, the meiofauna was extracted from the fixed samples using Ludox centrifugation and kinorhynchs were separated from other specimens under a stereomicroscope. Specimens for light microscopical (LM) studies were dehydrated through a graded glycerin series and mounted in Fluoromount G on glass slides. Specimens for LM were examined and photographed using Nomarski differential interference contrast (DIC) in an Olympus BX51 microscope equipped with an Olympus DP20 camera. Measurements were made with Cell^D software. Specimens for scanning electron microscopy (SEM) were dehydrated through a series of alcohol, and subsequently transferred to acetone through a graded alcohol/acetone series. When contained in 100% acetone, the specimens were critical point dried, mounted on aluminium stubs, sputter coated with a platinum/palladium mix and examined with a JEOL JSM-6335F Field Emission scanning electron microscope.

The terminology in the taxonomic account generally follows Neuhaus and Higgins (2002), Sørensen and Pardos (2008) and the most recent papers of the last author (Sørensen *et al.* 2012a, 2012b, in press). Terminology related to seta distribution for homalorhagids follows the standardization criteria establish by Sánchez *et al.* (2011) and adopted by most authors (Sørensen *et al.* 2010a, Yamasaki *et al.* 2012, Sánchez *et al.* 2013), except for Neuhaus (2012). The terminology tied to the introvert has been modified slightly, so all scalids (except trichoscalids) are referred to as spinoscalids, and those in Ring 01 as primary spinoscalids. This modification is made to make the terminology used by different experts more uniform. It is based on a suggestion made by Neuhaus (Neuhaus 2012), and a consensus about the future use of this terminology was recently reached at the Third International Scalidophora Workshop.

All type material is deposited at the Natural History Museum of Denmark (NHMD), whereas non-types are stored in the personal collection of the last author, and will later become integrated in the NHMD collection.

RESULTS

Order Homalorhagida (Zelinka, 1896)
Family Pycnophyidae Zelinka, 1896
Genus *Pycnophyes* Zelinka, 1907

Pycnophyes pardosi n. sp.
(Figs 2-4)

Type material. Holotype, adult male, collected on 6 October 2008 in the Korea Strait, locality MAP27 (Fig. 1A): 34°16′25″N, 128°40′24″E, from mud with tiny shells at 96 m depth; mounted in Fluoromount G®, deposited at the NHMD under accession number ZMUC KIN-638. Allotype, adult female, same collecting data as holotype, mounted in Fluoromount G®, deposited at NHMD under accession number ZMUC KIN-639. Paratypes, three adult males; two collected on same date and locality as holotype, mounted in Fluoromount G®, deposited at NHMD under accession numbers ZMUC KIN-603 and KIN-640; one collected on 6 August 2008 in the East China Sea, locality MAP25 (Fig. 1A) 28°32′41″N, 125°09′32″E, from deep sea mud at 104 m depth; mounted in Fluoromount G®, deposited at NHMD under accession number ZMUC KIN-641. Five additional specimens, mounted for SEM, are stored in the last author's collection and will later be included in the NHMD general collection: three specimens were collected on the same date and at the same locality as the holotype (one male and two females), one male was collected on 6 October 2008 in the Korea Strait, locality MAP28: 33°44′31″N, 128°15′234″E, from mud with tiny shells at 126 m depth; and one female on the same date in the Korea Strait, locality MAP29 32°37′29″N, 126°42′14″E, from mud with tiny shells at 128 m depth.

Diagnosis. Pycnophyes without middorsal structures (middorsal processes or elevations) or intracuticular atria on any segment. Single paradorsal seta present on segments 2-8 and one pair of paradorsal setae on segment 9. The single paradorsal setae may appear on either the right or left side of the middorsal line. Tergal anterior margin of segment 1 denticulated and ornamented, and posterior margin broadly triangular. Ventral anterior margin of segment 1 bulging and ornamented. Dorsal and ventral sides on segment 10 with longitudinal cuticular thickenings, visible with DIC and SEM. Type 1 sensory spots on segments 1-10 and males with type 3 sensory spots on segment 11.

Etymology. This species is named in honor of Prof. Fernando Pardos (Spain), who is the PhD supervisor of the first author and a good friend and colleague of the last author.

Description. None of the specimens were suitable for introvert examinations, so data on number and arrangement of scalids and oral teeth are not available.

Neck with four dorsal and two ventral placids (Fig. 3E). Dorsal placids are rectangular and of similar size, whereas the ventral ones are much more elongate and narrow towards the lateral sides. All placids appear robust and hard, with a depression on their surface (Figs 3E, 4E). They articulate with the anterior edge of the first trunk segment. Cuticular folds appear between dorsal and ventral placids. Trichoscalid plates not present.

Trunk with 11 segments (Figs 2A-B, 3A-C, 4A-B); first segment with one tergal and three ventral plates (Figs 2A, 4A, E). Segments 2-11 with one tergal and two ventral sternal plates. All dimensions and measurements of the examined specimens are summarized in Table 1, and distribution of sensory spots and setae in Table 2. The segment width is nearly constant throughout the trunk. Maximum width is reached at segment 6 and the posterior segments taper slightly from this segment. Posterior margins of segments with thin pectinate fringe, and a free flap overlapping the anterior edge of the following segment. In addition, all segments with dorsoventral muscles, visible as muscular scars in subdorsal and ventromedial positions. Several subepidermal granules grouped throughout the lateral margins of all segments, only visible with DIC (Fig. 4F). Dorsal and ventral pectinate fringes becoming more developed towards the lateral margins.

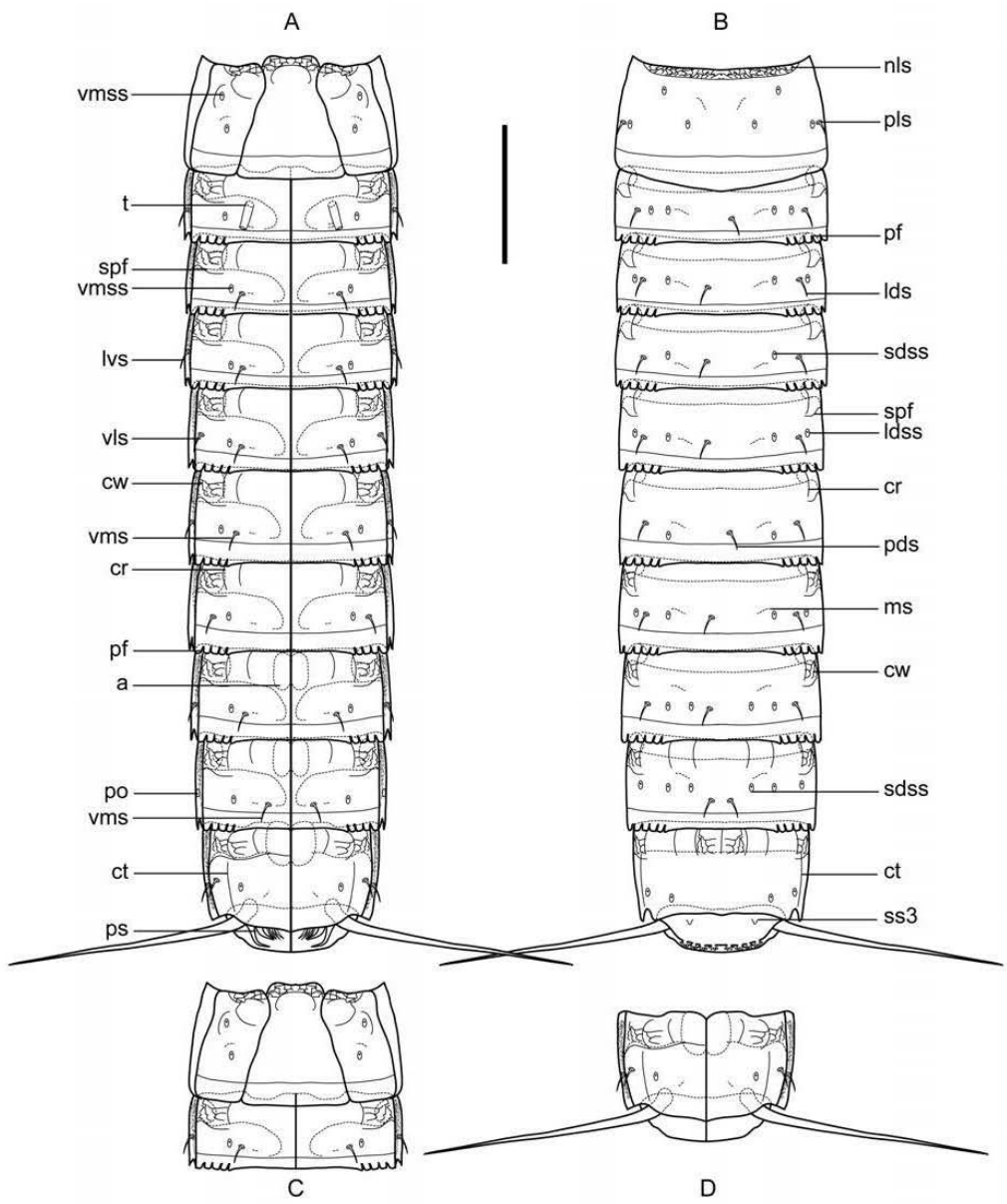

FIG. 2. – Line art illustrations of *Pycnophyes pardosi* n. sp. A, male, ventral view; B, male, dorsal view; C. female, segments 1-2, ventral view; D, female, segments 10-11, ventral view. Scale bar: 100 µm; *a* apodeme, *cr* cuticular ridge, *ct* cuticular thickening, *cw* cuticular wrinkles, *lds* laterodorsal seta, *ldss* laterodorsal sensory spot, *lvs* lateroventral seta, *ms* muscular scar, *nls* net-like structure, *pds* paradorsal seta, *pf* pectinate fringe, *pls* paralateral seta, *po* protonephridial opening, *ps* penile spine, *sdss* subdorsal sensory spot, *spf* secondary pectinate fringe, *ss3* sensory spot type 3, *t* tube, *vls* ventrolateral seta, *vms* ventromedial seta, *vmss* ventromedial sensory spot.

One pair of ventromedial cuticular ridges on segment 2 and two pairs on segments 3-10 (Fig. 3A). Pachycycli of tergal and sternal plates as well as peg and socket joints well developed from segments 2 to 8, and less conspicuous on segment 9-10 (Fig. 4A-B). Tergosternal junction on segments 2-10 covered by short cuticular hairs (Fig. 3K).

Segment 1: Anterolateral margins of tergal plate project into horn-like extensions. Tergal anterior margin of the segment strongly denticulated, followed by a

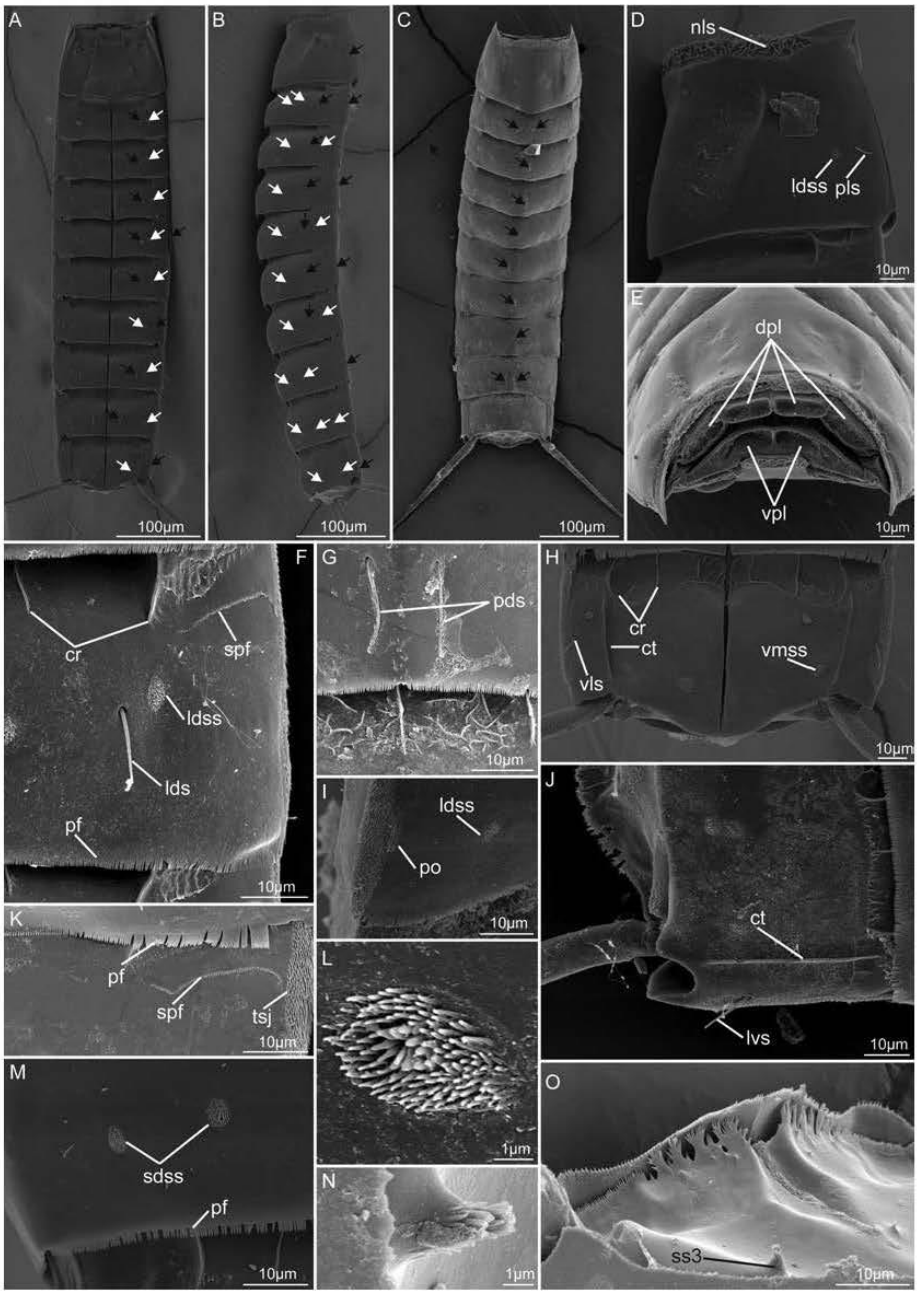

FIG. 3. – *Pycnophyes pardosi* n. sp. SEM photographs. A, female, ventral view; B, female, lateral view; C male, dorsal view; D, female, lateral view of segment 1; E, male, anterior view of segment 1, dorsal and ventral placids; F, female, dorsal view of right half of segment 3; G, male, dorsal view of paradorsal regions of segment 9; H, female, ventral view of segment 10; I, female, dorsolateral view of segment 9; J, female, dorsal view of right half of segment 10; K, male, detail in anterior laterodorsal position on segment 3; L, female, subdorsal sensory spot of segment 4; M, female, dorsal view of left half of segment 8; N, male, sensory spot type 3 of segment 11; O, male, dorsal view of segment 11, showing the special shape of the posterior fringe; *cr* cuticular ridge, *ct* cuticular thickening, *dpl* dorsal placid, *lds* laterodorsal seta, *ldss* laterodorsal sensory spot, *lvs* lateroventral seta, *nls* net-like structure, *pds* paradorsal seta, *pf* pectinate fringe, *pls* paralateral seta, *po* protonephridial opening, *sdss* subdorsal sensory spot, *spf* secondary pectinate fringe, *ss3* sensory spot type 3, *tsj* tergosternal junction, *vls* ventrolateral seta, *vmss* ventromedial sensory spot, *vpl* ventral placid. Sensory spots are marked with white arrows and setae with black arrows A-C.

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TABLE 1. – Measurements (µm) of adult *Pycnophyes pardosi* n. sp.; lts, lateral terminal spine; msw-6, maximum standard width of segment 6; n, number of measured specimens; SD, standard deviation; sw, standard width; s1-s11, segment lengths of trunk segments 1-11; TL, trunk length.

Character	n ♀	n ♂	Length allotype ♀	Range ♂	Average ♂	SD ♂
TL	1	4	676.7	604-667	633.3	29.8
sw10	1	4	121.3	117-131	125.9	5.8
sw/TL	1	4	0.18	0.19-0.20%	0.2%	0.004
msw-6	1	4	146.4	134-151	141.1	6.9
msw/TL	1	4	0.22	0.22-0.23%	0.22%	0.005
lts/TL	1	4	-	0.25-0.27%	0.26%	0.007
s1	1	4	99.4	79-100	89.0	7.8
s2	1	4	64.5	62-64	63.8	0.9
s3	1	4	62.5	61-62	62.8	2.0
s4	1	4	68.9	64-77	68.5	5.0
s5	1	4	66.9	65-77	69.0	5.5
s6	1	4	75.8	66-78	70.9	4.3
s7	1	4	73.9	67-78	71.5	4.2
s8	1	4	78.8	65-74	70.6	3.4
s9	1	4	81.7	65-80	71.8	6.5
s10	1	4	90.2	76-86	81.8	3.6
s11	-	-	-	-	-	-
lts11	-	2	-	152-174	164.2	16.8

TABLE 2. – Summary of location of setae and sensory spots in *Pycnophyes pardosi* n. sp. arranged by series. F, female condition of sexually dimorphic character; LD, laterodorsal; lts, lateral terminal spines; LV, lateroventral; m, male condition of sexually dimorphic character; ps, penile spines; PD, paradorsal; PL paralateral; SD, subdorsal; se, setae; * marks that the seta is unpaired; ss, sensory spots; ss3, sensory spot type 3; t, tubes; VL, ventrolateral; VM, ventromedial.

Segment	PD	SD	LD	PL	LV	VL	VM
1		ss,ss	ss	se			ss,ss
2	se*	ss	ss,se		se		ss,se(f),t(m)
3	se*	ss	ss,se				ss,se
4	se*	ss	se		se		ss,se
5	se*	ss	ss,se			se	ss,se
6	se*	ss	se		se		ss,se
7	se*	ss	ss,se				se,ss
8	se*	ss,ss	se		se		ss,se
9	se	ss,ss	ss				ss,se
10		ss	ss		se	se	ss
11		ss3(m)			lts	ps(2,m)	

characteristic cuticular ornamentation forming net-like structure (Fig. 3D). Posterior margin of dorsal plate slightly pointed middorsally, but without middorsal structure (middorsal process or elevation) or intracuticular atria (Fig. 3C). Tergal plate with one pair of muscular scars in the middle region, one pair of paralateral setae and three pairs of sensory spots, two pairs in subdorsal and one pair in laterodorsal positions, near the paralateral seta (Figs 2B, 3D). Sensory spots on this and all the following segments (except on segment 11) belong to type 1, are rounded to elongate and consist of several small cuticular papillae arranged around a collar of 8-10 wider papillae/petals with a cilium emerging through a central pore (similar to Fig. 3L). The sensory spots have one additional pore located outside the collar of papillae. Ventral side with two episternal plates and one trapezoidal midsternal plate, slightly longer than both episternal plates and hence overhanging the anterior edge of the segment (Fig. 4E). Episternal plates with a muscular scar in the middle region. Ventral anterior margin appears bulged and ornamented, followed by a contiguous depressed area. Each episternal plate with two ventromedial sensory spots. Pectinate fringe on both dorsal and ventral sides thin, but slightly more developed towards the lateral sides.

Segment 2: Tergal plate with a single paradorsal seta without associated middorsal structures (middorsal processes or elevations) or intracuticular atria (Fig. 2B). One specimen showed a pair of paradorsal setae (Fig. 3C), otherwise all other examined specimens showed only a single, unpaired seta in either left or right side paradorsal position. A pair of laterodorsal and lateroventral setae present. Two pairs of sensory spots, one subdorsal and one laterodorsal, present, the latter being close to the laterodorsal setae (Figs 3B, 4B). A pair of dorsoventral muscular scars appears on this and all the following segments until segment 10. Dorsal side with short secondary pectinate fringes in the anterior part of the segment, present from the tergosternal junction to the laterodorsal longitudinal ridge (Fig. 3K). Sternal plates with one pair of ventromedial sensory spots. Males always with a pair of large tubes located in the ventromedial position (Fig. 2A) and females with a pair of ventromedial setae (Fig. 2C). Ventral side with secondary pectinate fringe similar to that on the dorsal side, from the tergosternal junction to the first ventromedial cuticular ridge. Cuticular wrinkles located in the anteriormost parts of the sternal plates, from the tergosternal junction to the cuticular ridge, anterior to secondary pectinate fringe (Fig. 2A). Hairy tergosternal junctions, with numerous short cuticular hairs present (Fig. 3K). Pectinate fringe on both dorsal and ventral sides most developed near the lateral margins (Fig. 3K). Glandular cell outlets present anterolaterally on tergal and sternal plates, anterior to the secondary pectinate fringes. Pachycycli of tergal and sternal plates well developed, with a regular size of peg and socket joints.

Segment 3: Tergal plate with a single paradorsal seta but without middorsal structures (middorsal processes or elevations) or intracuticular atria, one pair of laterodorsal setae, and one pair of subdorsal and laterodorsal sensory spots (Figs 3B-C, 4B). Laterodorsal sensory spots more lateral than laterodorsal setae (Fig. 3F). Lateroventral setae not present. Sternal plates with one pair of ventromedial setae and slightly more lateral sensory spots (Fig. 3A). Tergosternal junctions, pachycycli, peg and socket joints, muscular scars, glandular cell outlets, cuticular wrinkles, pectinate fringe and secondary pectinate fringes as on the preceding segment.

Segment 4: Tergal plate with a single paradorsal seta but without middorsal structures (middorsal processes or elevations) or intracuticular atria (Figs 2B, 4B). Additional setae include one pair in laterodorsal and in lateroventral positions. Paired sensory spots present in subdorsal positions (Figs 3B-C, 4B). Sternal plates same as on segment 3 (Figs 3A), and segment otherwise similar to segment 2.

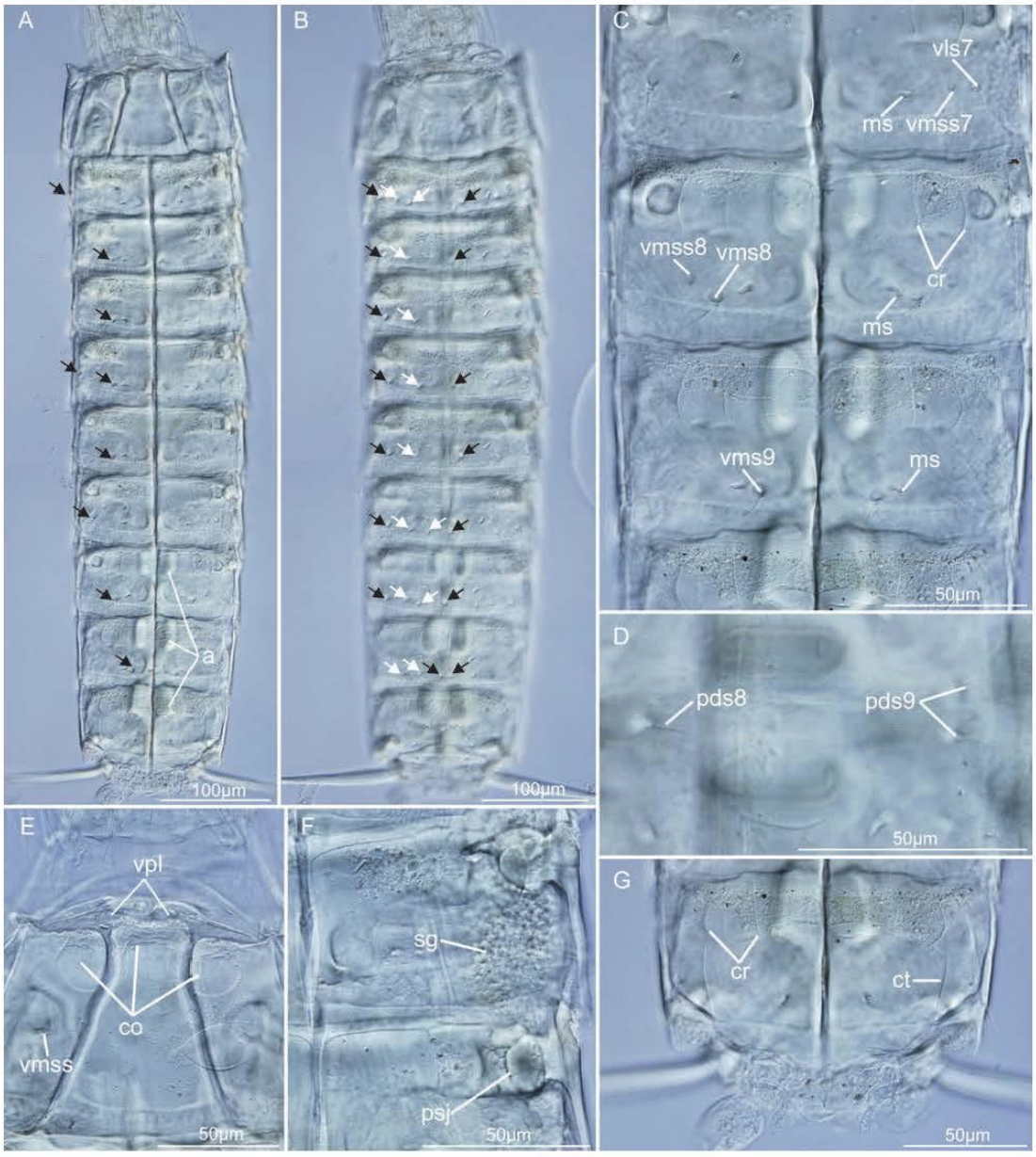


FIG. 4. – *Pycnophyes pardosi* n. sp. Differential interference contrast photographs of a male. A, ventral view; B, dorsal view; C, ventral view of segments 7-9; D, dorsal view of segments 8-9; E, ventral view of segment 1; F, ventral view of segments 3-4; G, ventral view of segment 10; *a* apodeme, *co* cuticular ornamentation, *cr* cuticular ridge, *ct* cuticular thickening, *ms* muscular scar, *pds* paradorsal seta, *psj* peg and socket joint, *sg* subcuticular gland, *vls* ventrolateral seta, *vms* ventromedial seta, *vmss* ventromedial sensory spot, *vpl* ventral placid. Sensory spots are marked with white arrows. Setae are marked with black arrows on A-B. Digits following labels refer to segment numbers.

Segment 5: Same as segment 3, but with a pair of ventrolateral setae (Figs 3A-B, 4A-B).

Segment 6: Same as segment 4 (Figs 3A-B, 4A-B).

Segment 7: Tergal plate similar to that on segment 3, but with cuticular wrinkles in the anteriormost region, from the tergosternal junction to the cuticular ridge, anterior to the secondary pectinate fringe. Sternal plates with paired sensory spots and setae, but, unlike preceding segments, with the setae located more laterally (Figs 3A, 4A, C). Otherwise similar to preceding segments.

Segment 8: Tergal plate with a single paradorsal seta without associated middorsal structures (middorsal processes or elevations) or intracuticular atria (Figs 3C, 4D). Paired laterodorsal setae were observed in eight out of the ten specimens, whereas paired lateroventral setae were present in all. Two pairs of subdorsal sensory spots present (Figs 3M, 4B). Cuticular wrinkles as on segment 7. Sternal plates as those on segment 3 (Fig. 4C). Paired apodemes (or anteromesial thickenings of ventral pachycycli) near anterior margin of segment were observed in three males (Fig. 2A). Otherwise similar to preceding segments.

Segment 9: Tergal plate with one pair of paradorsal setae without middorsal structures (middorsal processes or elevations) or intracuticular atria (Figs 3C, G, 4D). Three pairs of sensory spots present, two in subdorsal and one in laterodorsal positions (Figs 3B, I, 4B). Cuticular wrinkles as on segment 7. Protonephridial opening in paralateral position, with the pore surrounded by a few short cuticular hairs; opening not sieve-like (Fig. 3I). Sternal plates with one pair of ventromedial sensory spots and setae, the latter located close to the limit of the paraventral position (Fig. 4C). Paired apodemes present near anterior margin of segment. Peg and socket joints inconspicuous. Otherwise similar to preceding segment.

Segment 10: Tergal plate with one pair of lateroventral setae and pairs of subdorsal and laterodorsal sensory spots near the posterior segment margin (Fig. 3B). Middorsal structure (middorsal process or elevation) or intracuticular atria absent. Cuticular wrinkles on the lateral margins as on segment 7, but also present in the subdorsal region (Fig. 2B). Lateral margins, furthermore, with longitudinal cuticular thickening visible with DIC and SEM (Fig. 3J). Sternal plates also with somewhat similar cuticular thickenings near their lateral margins (Figs 3H, 4G). Ventrolateral setae and ventromedial sensory spots present near the longitudinal hardening (Fig. 3H). A pair of apodemes present near the anterior margin. Peg and socket joints inconspicuous. Otherwise similar to preceding segments.

Segment 11: With paired lateral terminal spines (Figs 2A-B, D, 3C). Males with two pairs of penile spines and genital pores surrounded by a tuft of long hairs (Fig. 2A). Tergal plate with paired type 3 sensory spots, present in males only (Fig. 3N-O). Posterior segment margin with characteristic, irregularly fringed shape (see Fig. 3O).

Associated kinorhynch fauna. Pycnophyes pardosi n. sp. co-occurs with *Echinoderes tchefouensis* Lou, 1934 and other indetermined *Echinoderes* spp. at all four localities. Undetermined species of *Condyloderes* sp. were furthermore recorded from three localities, MAP25, 27 and 29. At localities MAP28 and 29 it also co-occurs with *E. microaperturus* Sørensen *et al.*, 2012 and *Meristoderes elleae* Sørensen *et al.*, in press, and at MAP25 with *Dracoderes abei* (see also Sørensen *et al.* 2012a, 2012b, in press).

Remarks. Pycnophyes pardosi n. sp. is easily distinguished from the only four species of the genus known from the region, *P. furugelmi*, *P. oshoroensis*, *P. schornikovi*, and *P. tubuliferus* (see Adrianov 1989, Adrianov and Malakhov 1999, Yamasaki *et al.* 2012), as none of them have paradorsal setae on all segments from 2 to 9.

Several other species, though, share the presence of paradorsal or middorsal setae on segments 2-9 with *Pycnophyes pardosi* n. sp. These include *P. australensis* Lemburg, 2002; *P. carinatus* Zelinka, 1928; *P. corrugatus* Higgins, 1983; *P. dentatus* Zelinka, 1928; *P. egyptensis* Higgins, 1966; *P. faveolus* Brown, 1999 (in Adrianov and Malakhov 1999); *P. flaveolatus* Zelinka, 1928; *P. kielensis* Zelinka, 1928; *P. newguiniensis* Adrianov, 1999; *P. parasanjuanensis* Adrianov and Higgins, 1996; *P. robustus* Zelinka, 1928 and *P. sanjuanensis* Higgins, 1961 (see Zelinka 1928, Higgins 1961, 1966, Brown 1985, Adrianov and Higgins 1996, Adrianov and Malakhov 1999, Lemburg 2002). However, whereas the presence of paradorsal setae is rather common, only six of these species have at least one segment with paired paradorsal setae. These species are *P. carinatus*, *P. faveolus*, *P. kielensis*, *P. newguiniensis*, *P. parasanjuanensis* and *P. robustus* (see Zelinka 1928, Brown 1985, Adrianov and Higgins 1996, Adrianov and Malakhov 1999). The new species can be distinguished from *P. carinatus*, *P. faveolus* and *P. newguiniensis* by its absence of middorsal or paradorsal setae on segment 1. Furthermore, *P. carinatus* has pairs of paradorsal setae on segment 1 (Zelinka 1928), *P. faveolus* on segments 3, 5 and 7-9 (Brown 1985), and *P. newguiniensis* on segments 2-9 (Adrianov and Malakhov 1999). The remaining three species also have pairs of paradorsal setae on other segments than those found in *Pycnophyes pardosi* n. sp. *Pycnophyes kielensis* has a single paradorsal seta on segment 8 only (Zelinka 1928), *P. parasanjuanensis* has a pair of paradorsal seta on segment 6 (males with another pair on segment 2) (Adrianov and Higgins 1996) and *P. robustus* on segments 3, 5, 7 and 9 (Zelinka 1928). Moreover, these six species do not have longitudinal cuticular thickenings on the dorsal and ventral sides on segment 10.

Since many old, but also some more recent, descriptions of species do not pay enough attention to the sensory spot distribution, it is not always helpful to use these for comparison of species. Of the species discussed above, only the description of *P. faveolus* provides accurate information on sensory spot distribution (Brown 1985).

The presence of cuticular ornamentation as a net-like structure on the dorsal anterior margin of segment 1, as present in the new species, is shared with *P. dentatus*, *P. lageria* Sánchez *et al.*, 2013; *P. faveolus*, *P. furugelmi*, *P. oshoroensis*, *P. parasanjuanensis* and *P. sanjuanensis* (see Zelinka 1928, Higgins 1961, Brown 1985, Adrianov and Higgins 1996, Adrianov and Malakhov 1999, Yamasaki *et al.* 2012, Sánchez

et al. 2013). Out of these, only *P. dentatus* and *P. lageria* also present cuticular ornamentation on the lateral margins of segment 10 (see Zelinka 1928, Sánchez *et al*. 2013). However, *Pycnophyes pardosi* n. sp. has a pair of longitudinal cuticular thickenings on segment 10, whereas *P. dentatus* (Zelinka 1928) has a higher number of longitudinal thickenings or vertical cuticular striations on this segment, and *P. lageria* has circular depressions on the same segment (Sánchez *et al*. 2013).

Pycnophyes chalgap n. sp.
(Figs 5-8)

Type material: Holotype, adult male, collected on 26 May 2008, at the Korean south coast, near Yeosu, locality MAP13 (Fig. 1B) 34°42′17″N, 127°43′01″E, from mud at 4 m depth; mounted in Fluoromount G®, deposited at the NHMD, under accession number ZMUC KIN-642. No females were available, hence no allotype is appointed. Paratypes, two adult males; one collected on same date and locality as holotype and one also on 26 May 2008 at a nearby locality, MAP15 (Fig. 1B) 34°34′43″N, 127°45′52″E, from mud with shell gravel at 13 m depth. Paratypes are mounted in Fluoromount G® and deposited at the NHMD under accession numbers ZMUC KIN-643 and KIN-644, respectively. Two additional male specimens mounted for SEM, one collected at locality MAP13 together with the holotype and one from MAP15, together with the paratype. Both specimens are stored in the last author's collection and will later be included in the NHMD general collection.

Diagnosis. *Pycnophyes* with the posterior margin of the midsternal plate rounded and longer than both episternal plates, extending beyond the anterior half of the following segment. Ventral anterior margin of segment 1 bulged and ornamented. Tergal anterior margin of segment 1 with fine denticulation and with a contiguous ornamented semicircular area. Middorsal bulge present on segments 2-4, whereas no middorsal processes or elevations are present on the remaining segments. Intracuticular atria visible with DIC on segments 2-9. Single paradorsal setae present on segments 2, 4, 6 and 8. Type 1 sensory spots present on segments 1-10, and type 3 sensory spots on segment 11 but in males only.

Etymology. The species name, *chalgap*, refers to a unit in the traditional Korean warrior armor that is used to protect the neck and throat. The name is inspired by the shape of the extended midsternal plate on segment 1.

Description. Mouth cone cylindrical and smooth, without any conspicuous cuticular features. Nine equally sized outer oral styles present, being thin, long, flexible and unarticulated, located one by each introvert sector except for the absence of an oral style in the middorsal sector 6 (Fig. 6).

The introvert has six rings of spinoscalids and one additional ring of trichoscalids (Figs 6, 7K). Ring 01 has ten primary spinoscalids consisting of two pieces: a short basal sheath and a long distal end. The basal piece of each primary spinoscalid is equipped with a row of long fringes, longer in the middle region of the spinoscalid than on the lateral margins. Ring 02 is formed by ten spinoscalids, all consisting of a long end piece and a basal sheath. The basal sheath of each spinoscalid appears hairy at its proximal end and terminates into a short fringe. Ring 03 with 20 spinoscalids, two in each introvert sector, all resembling those of ring 02 (Fig. 6). Ring 04 is formed by only five spinoscalids, one in each odd numbered sector, and similar to those of the preceding rings but with a long fringe at the base. Ring 05 with 15 spinoscalids, one in each even numbered sector and two in odd numbered ones, and ring 06 with 13 spinoscalids, one in even numbered sectors and two in odd numbered ones except sector 6, where ring 06 spinoscalids are missing. Spinoscalids of rings 05 and 06 resemble those of ring 04. Ring 07 has 14 trichoscalids attached directly to the introvert, not through trichoscalid plates. All trichoscalids with a wide and hairy base and a narrower terminal tip.

Described by sector (Fig. 6), the spinoscalids are arranged in two groups, each one composed of a central scalid anterior to a pair scalids. The anterior and posterior scalid triplet is separated by ring 04, which has no scalids in even numbered sectors. Even sectors have six spinoscalids and one trichoscalid. The middorsal sector 6 has only the anterior scalid triplet and a single, central posterior scalid. Odd numbered sectors have seven spinoscalids and two trichoscalids, except sector 1 with only one trichoscalid. Scalids are arranged as a "double diamond" in each sector. See Figure 6 for a complete summary of oral styles and scalid and placid locations.

Neck with four dorsal and two ventral placids, all robust and hardly sclerotized. Dorsal placids are rectangular, whereas ventral placids have a more rounded appearance (Fig. 5A-B). All placids articulate with the first trunk segment and extend anteriorly along the thin and flexible cuticle of the introvert. Cuticular folds are present between the dorsal and ventral placids.

Trunk composed of 11 segments, with first segment composed of one tergal and three ventral plates (Figs 5A-B, 7A-B, D, 8A-C). Segments 2-11 with one tergal and two sternal plates. All dimensions and measures are summarized in Table 3, and distribution of sensory spots and setae in Table 4. The segment width is nearly constant throughout the trunk. Maximum sternal width is reached on segment 3 and the segments taper slightly from this segment. Most segment surfaces are covered by cuticular hairs and the tergosternal junctions on segments 2-10 appear hairy. Bulging middorsal region present on segments 2-4 (Figs 5B, 7B-C, 8D, G). Anteriormost region of both dorsal and ventral sides with a cuticular ornamentation on segments 2-10, more developed on segments 8-10. One pair of laterodorsal cuticular ridges on segments 2-6, ventromedial ones on segments 2-7 and two pairs on segments 8-10 (Fig. 5A-B). Subdorsal and ventromedial muscular scars present on each segment (Fig. 5A-B). One pair of apodemes near anterior margin on segments 5-10 (Fig. 8A).

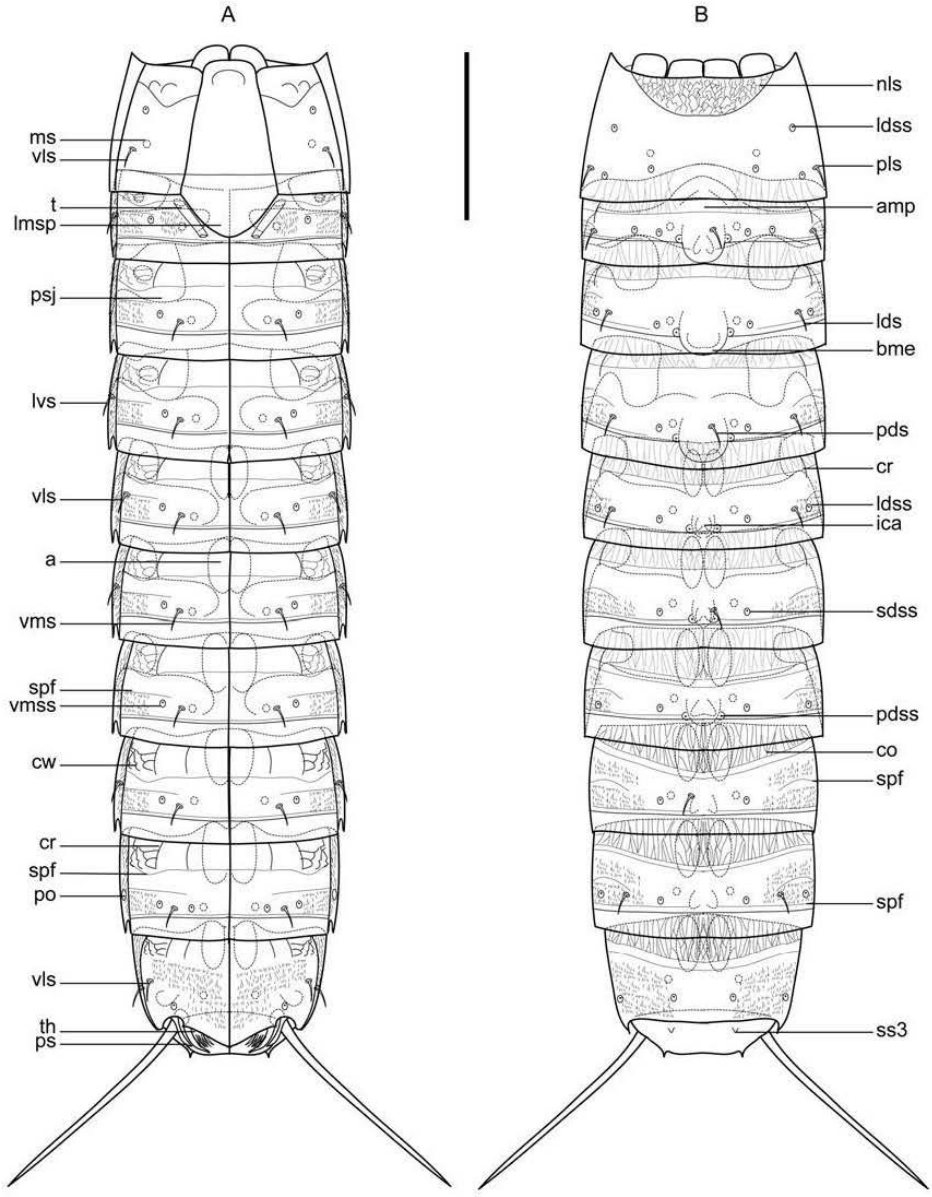
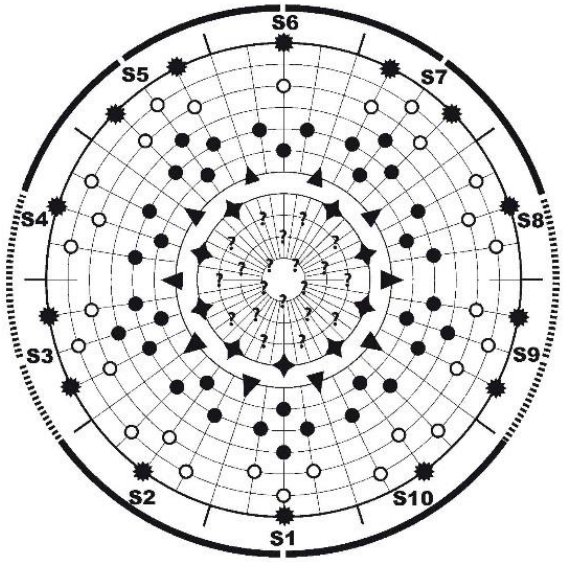


FIG. 5. – Line art illustrations of *Pycnophyes chalgap* n. sp. A, male, ventral view; B, male, dorsal view. Scale bar: 100 µm; *a* apodeme, *amp* anterior middorsal projection, *bme* bulge middorsal elevation, *co* cuticular ornamentation, *cr* cuticular ridge, *cw* cuticular wrinkles, *ica* intracuticular atria of sensory spot, *lds* laterodorsal seta, *ldss* laterodorsal sensory spot, *lmsp* long midsternal plate, *lvs* lateroventral seta, *ms* muscular scar, *nls* net-like structure, *pds* paradorsal seta, *pdss* paradorsal sensory spot, *pls* paralateral seta, *po* protonephridial opening, *ps* penile spine, *psj* peg and socket joint, *sdss* subdorsal sensory spot, *spf* secondary pectinate fringe, *ss3* sensory spot type 3, *t* tube, *th* tuft of hairs, *vls* ventrolateral seta, *vms* ventromedial seta, *vmss* ventromedial sensory spot.

Pachycycli of tergal and sternal plates from segments 2-7 well developed, regular sized peg and socket joints present on segments 2 and 5-7 and more developed ones on segments 3-4. Pachycycli on the remaining segments not visible (Figs 5, 8A).

Segment 1: Anterolateral margins of tergal plate slightly projecting into horn-like extensions. Characteristic semicircular cuticular ornamentation appears as net-like structure on anterior fourth of tergal plate (Figs 5B, 8D). Middorsal structures (middorsal process



Scalid and style arrangement

Ring/Sector		1	2	3	4	5	6	7	8	9	10	Total
00 oos	✦	1	1	1	1	1	0	1	1	1	1	9
01	▼	1	1	1	1	1	1	1	1	1	1	10
02	●	1	1	1	1	1	1	1	1	1	1	10
03	●	2	2	2	2	2	2	2	2	2	2	20
04	●	1	0	1	0	1	0	1	0	1	0	5
05	○	2	1	2	1	2	1	2	1	2	1	15
06	○	1	2	1	2	1	0	1	2	1	2	13
07 tr	✸	1	1	2	1	2	1	2	1	2	1	14
Total scalids		8	7	9	7	9	5	9	7	9	7	87

FIG. 6. – Diagram of mouth cone, introvert and placids in *Pycnophyes chalgap* n. sp. with the distribution and type of scalids by ring and sector. Dashed lines mark flexible cuticular lateral areas. "Double diamonds" are marked in the table with double lines; *oos* outer oral style, *tr* trichoscalid, *s1-s10* sector number.

or middorsal elevation) or intracuticular atria not present. Tergal plate with one pair of subdorsal muscular scars in the middle region of the segment, one pair of paralateral setae and three pairs of sensory spots, one in subdorsal and two in laterodorsal positions, the latter near the paralateral seta (Figs 5B, 7B). Sensory spots on this and all following nine segments belong to type 1, being rounded to elongate and consisting of many small cuticular papillae surrounding a pore. Ventral side with two episternal plates and one midsternal plate. Midsternal plate with rounded, posterior extension that projects beyond the margin of the episternal plates and overlaps the anterior half of the following segment (Figs 7A, D, F, 8C). Muscular scar present in the middle region of the episternal plates. Anterior margin of ventral side bulged and ornamented, with a more posterior contiguous depressed area (Figs 7D, 8C). Each episternal plate with one ventrolateral seta and one ventromedial sensory spot (Figs 7D, 8A, C). Pectinate fringes serrated, only visible with SEM.

Segment 2: Tergal plate with a single paradorsal seta, one pair of laterodorsal and lateroventral setae and one pair of paradorsal sensory spots associated with intracuticular atria (Figs 5B, 8B). This pair of sensory spots appears closer to the middle region of the segment than to the posterior margin. Two additional pairs of sensory spots present in subdorsal positions (Fig. 8B). Blunt anterior middorsal projection present. Middorsal region from this segment to segment 4 appears very high or bulging, hump-like (Figs 7B, 8D). Three secondary pectinate fringes present parallel to the anterior margin of the segment (Fig. 5B). The anteriormost fringe stretches over the tergal plate, whereas the second one extends from the lateral margins to the laterodorsal setae, where it is V-shaped and covers the insertion of the seta (similar to Fig. 7E). The posteriormost fringe extends from the lateral margins to the subdorsal position. Short cuticular hairs, not arranged in any particular pattern, are present between these secondary fringes, from the lateral margins to the laterodorsal position. One pair of subdorsal muscular scars appears on this and all following segments (except on segment 11). Sternal plates with a pair of ventromedial sensory spots (Figs 5A, 7A, F). Males with a pair of large, ven-

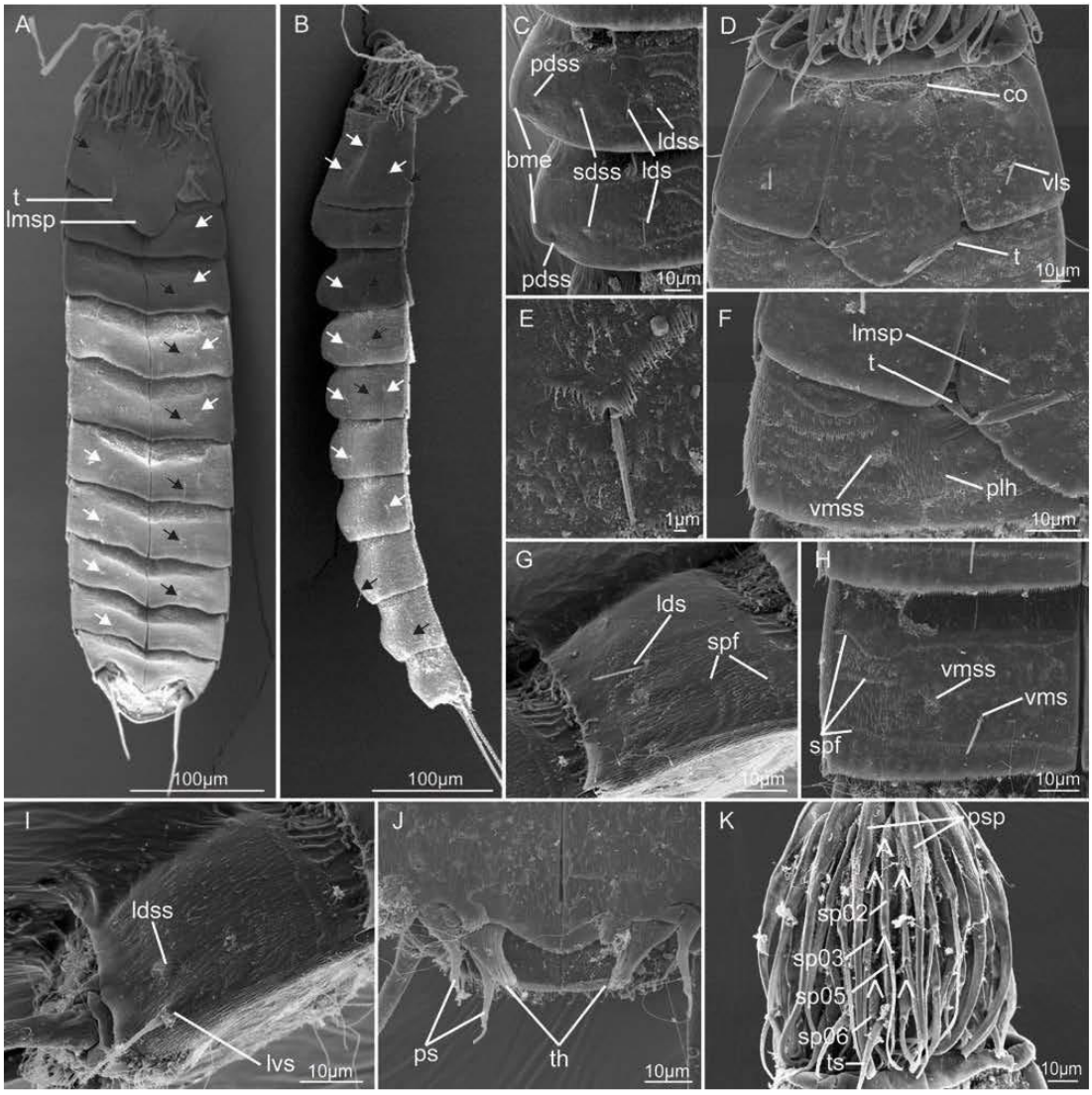


FIG. 7. – *Pycnophyes chalgap* n. sp. SEM photographs of male. A, ventral view; B, lateral view; C, dorsolateral view of segments 3-4; D, ventral view of segment 1; E, laterodorsal seta on segment 4; F, ventral view of segments 1-2; G, lateral view of segment 9; H, right sternal plate of segment 7; I, lateral view of segment 9; J, ventral view of segments 10-11; K, introvert sectors 1-2; *bme* bulge middorsal elevation, *co* cuticular ornamentation, *lds* laterodorsal seta, *ldss* laterodorsal sensory spot, *lmsp* long midsternal plate, *lvs* lateroventral seta, *pdss* paradorsal sensory spot, *plh* patch of long hairs, *ps* penile spine, *psp* primary spinoscalid, *sdss* subdorsal sensory spot, *sp* spinoscalid, *spf* secondary pectinate fringe, *t* tube, *th* tuft of hairs, *ts* trichoscalid, *vls* ventrolateral seta, *vms* ventromedial seta, *vmss* ventromedial sensory spot. Sensory spots are marked with white arrows and setae with black arrows on A-B. Digits following labels on K refer to the introvert ring numbers. Lambda symbols (Λ) mark attachment points of scalids.

tromedial tubes (Fig. 7F). Tergosternal junctions hairy. Three secondary pectinate fringes on the ventral side: two in the anterior region of the sternal plates from the tergosternal junctions to the ventromedial cuticular ridges, and the anteriormost stretching across the complete surface of the sternal plates (Fig. 5A). Patch of longer hairs located in ventromedial position and additional patches of short cuticular hairs present in the ventrolateral region between the secondary fringes (Fig. 7F). Pectinate fringes slightly less developed in the dorsal and ventrolateral areas than in ventromedial and paraventral ones. Glandular cell outlets present on tergal and sternal plates, between anterolateral margin of the segment and the anteriormost secondary pectinate fringes. One pair of ventromedial muscular scars appears on this and all following segments. Pachycycli

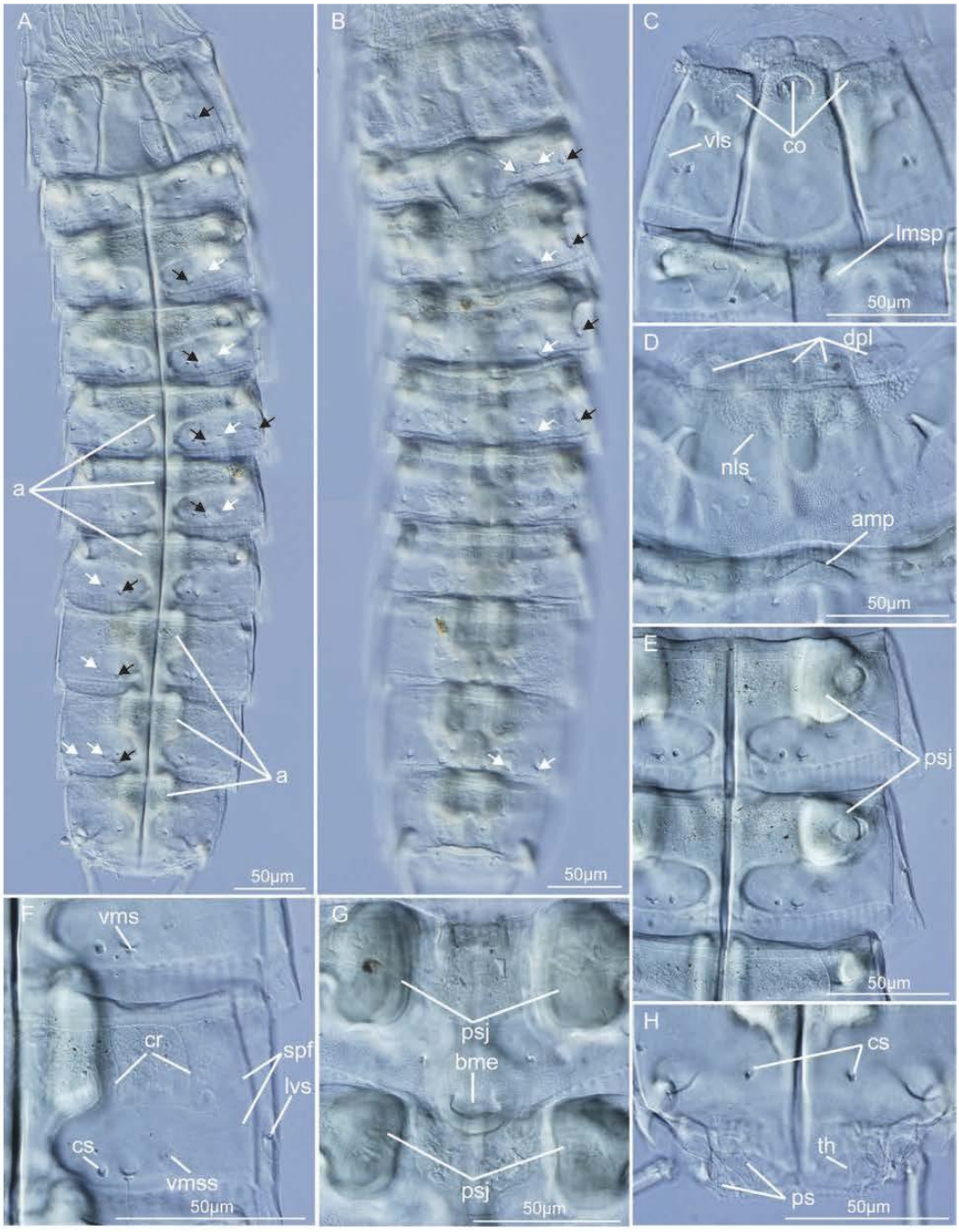


FIG. 8. – *Pycnophyes chalgap* n. sp. Differential interference contrast photographs of male. A, ventral view; B, dorsal view; C, central view segments 1-2; D, dorsal view of segments 1-2; E, ventral view of segments 3-4; F, right sternal plate of segment 8; G, dorsal view of segments 3-4; H, ventral view of segments 10-11; *a* apodeme, *amp* anterior middorsal projection, *bme* bulge middorsal elevation, *co* cuticular ornamentation, *cr* cuticular ridge, *cs* cuticular scar, *dpl* dorsal placid, *lmsp* long midsternal plate, *lvs* lateroventral seta, *nls* net-like structure, *ps* penile spine, *psj* peg and socket joint, *spf* secondary pectinate fringe, *th* tuft of hairs, *vls* ventrolateral seta, *vms* ventromedial seta, *vmss* ventromedial sensory spot. Sensory spots are marked with white arrows and setae with black arrows on A-B.

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TABLE 3. – Measurements (μm) of adult *Pycnophyes chalgap* n. sp.; esp, episternal plate; lts, lateral terminal spine; msp, midsternal plate; msw-3, maximum standard width of segment 3; n, number of measured specimens; SD, standard deviation; sw, standard width; s1-s11, segment lengths of trunk segments 1-11; TL, trunk length.

Character	n ♂	Range ♂	Average ♂	SD ♂
TL	3	573-619	596.4	23.1
sw10	3	100-114	105.7	7.7
sw/TL	3	0.17-0.18%	0.18%	0.009
msw-3	3	129-151	138.1	11.8
msw/TL	3	0.22-0.24%	0.23%	0.01
lts/TL	1	0.27%	0.27%	-
esp/msp	3	0.73-0.75%	0.75%	0.02
msp	3	100-105	102.6	2.6
esp	3	76-77	76.7	0.5
s2	3	32-46	39.7	7.5
s3	3	64-70	66.4	3.4
s4	3	66-70	67.8	1.7
s5	3	61-73	65.8	6.3
s6	3	62-68	65.8	3.0
s7	3	62-67	64.9	2.9
s8	3	68-74	70.1	3.3
s9	3	65-70	67.3	2.9
s10	3	70-77	72.9	3.9
s11	3	-	-	-
lts11	1	165	165	-

of tergal and sternal plates well developed, with normal sized the peg and socket joints (Fig. 8**A**).

Segment 3: Tergal plate with hump-like middorsal region (Fig. 7B-C). One pair of paradorsal sensory spots associated with intracuticular atria. Paradorsal sensory spots located as on segment 2. Two additional pairs of sensory spots present in subdorsal and laterodorsal positions. One pair of laterodorsal setae located mesially to the laterodorsal sensory spots (Fig. 7C). Sternal plates with one pair of ventromedial setae and sensory spots, the latter located more laterally (Figs 7**A**, 8**A**). With ventrolateral cuticular hair patches. Dorsal and ventral sides with cuticular wrinkles on the anteriormost area on this and all following segments (except segment 11). Pachycycli and peg and socket joints much more developed than on preceding segment (Fig. 8**A**, E, G). Tergosternal junctions, glandular cell outlets, pectinate fringe and secondary fringes similar to those on preceding segment.

Segment 4: Tergal plate similar to that on segment 3 but without laterodorsal sensory spots, and with a single paradorsal seta and a pair of lateroventral setae (Figs 5B, 7B, 8B). Sternal plates, pachycycli, peg and socket joints, and other structures similar to those on segment 3 (Fig. 8**A**, E, G).

Segment 5: Tergal and sternal plate similar to those on segment 3, but without the middorsal bulge and with a pair of ventrolateral setae (Figs 7**A**-B, 8**A**-B). Pachycycli and peg and socket joints similar to those on segment 2. One pair of apodemes present near anterior margin of the segment (Figs 5**A**, 8**A**). Otherwise similar to preceding segments.

Segment 6: Tergal plate with a single paradorsal seta and one pair of paradorsal sensory spots associated with intracuticular atria (Figs 5B, 7B). Pairs of subdorsal sensory spots and lateroventral setae present. Sternal plates similar to those on segment 3 with one pair of apodemes near anterior margin of the segment. Pachycycli and peg and socket joints similar to those on segment 2. Otherwise similar to preceding segments.

TABLE 4. – Summary of location of setae and sensory spots in *Pycnophyes chalgap* n. sp. arranged by series. LD, laterodorsal; lts, lateral terminal spines; LV, lateroventral; m, character trait assumed to be male condition of sexually dimorphic character; ps, penile spines; PD, paradorsal; PL, paralateral; SD, subdorsal; se, setae; * marks that the seta is unpaired; ss, sensory spots; ss3, sensory spot type 3; t, tubes; VL, ventrolateral; VM, ventromedial.

Segment	PD	SD	LD	PL	LV	VL	VM
1		ss	ss,ss	se		se	ss
2	se*,ss	ss,ss	se		se		ss,t(m)
3	ss	ss	ss,se				ss,se
4	se*,ss	ss	se		se		ss,se
5	ss	ss	ss,se			se	ss,se
6	se*,ss	ss			se		ss,se
7	ss	ss	ss				ss,se
8	se*	ss			se		ss,se
9		ss	se,ss				ss,se,ss
10		ss	ss		se	se	ss
11		ss3(m)			lts	ps(2,m)	

Segment 7: Tergal and sternal plates similar to those on segment 3 (Fig. 7H), except for the absence of laterodorsal setae and the middorsal bulge. One pair of apodemes present near anterior margin of segment. Pachycycli and peg and socket joints similar to those on segment 2. Otherwise similar on preceding segments.

Segment 8: Tergal plate similar to that on segment 6, but with the single paradorsal seta located on the opposite side and an apparent lack of paradorsal sensory spots (Fig. 5B). Lateroventral setae not present. One specimen with paired laterodorsal setae was observed. Sternal plates similar to those on segment 3, but with an additional pair of ventromedial cuticular ridges and some additional hairs that may appear in the ventromedial position (Fig. 8F). Dorsal and ventral sides with the cuticular ornamentation in the anteriormost area of the segment more developed than on preceding segments. Pachycycli and peg and socket joints are not visible (Fig. 8**A**, F). Otherwise similar to preceding segments.

Segment 9: Tergal plate similar to that on segment 5, except for the apparent lack of paradorsal sensory spots (Fig. 7G). Protonephridial opening paralaterally, not sieve-like, surrounded by several short hairs. Sternal plates similar to those on segment 8, but with an additional pair of ventromedial sensory spots (Fig. 8**A**). Cuticular ornamentation on dorsal and ventral sides as on segment 8. Pachycycli and peg and socket joints not visible. Otherwise similar to preceding segments.

Segment 10: Tergal plate with one pair of lateroventral setae and one pair of subdorsal and laterodorsal sensory spots (Fig. 7I). Middorsal structure (middorsal process or elevation) or intracuticular atria absent. Sternal plates with one pair of ventrolateral setae and one pair of ventromedial sensory spots close to the posterior margin of the segment. Cuticular hairs present throughout the surface of the segment (Fig. 7I-J). Cu-

ticular ornamentation on both dorsal and ventral sides as on segment 8. Pachycycli and peg and socket joints not visible. Otherwise similar to preceding segments.

Segment 11: With one pair of lateral terminal spines (Figs 5A-B, 7A-B). Tergal plate with two pairs of type 3 sensory spots in subdorsal positions (Fig. 5B). Cuticular hairs present throughout the surface of the segment. Males with two pairs of penile spines and two genital pores surrounded by a tuft of long hairs and one pair of cuticular extensions appearing somewhat horn-like (Figs 5A, 7J, 8H).

Associated kinorhynch fauna. Pycnophyes chalgap n. sp. lives in coastal waters at relatively shallow depths. Other species recorded from MAP13, the locality situated at 4 m depth, include *Kinorhynchus yushini* and *Meristoderes herranzae* Sørensen *et al.*, in press, whereas co-occurring species on the slightly deeper MAP15 locality include *Dracoderes abei*, *Echinoderes rex* Lundbye *et al.* 2011, *E. tchefouensis* and *K. yushini* (see Sørensen *et al.* 2012a, in press).

Remarks. Pycnophyes chalgap n. sp. is easily distinguished from the known species by the shape of its midsternal plate with the posterior margin being rounded and longer than both episternal plates, overlapping the anterior half of the following segment. This new species can also be recognized by its tergal anterior margin of segment 1 with a semicircular cuticular ornamentation as a net-like structure, apodemes on segments 5-10, peg and socket joints being more developed on segments 3-4 than on other segments, middorsal bulge on segments 2-4 and the presence of single paradorsal setae on segments 2, 4, 6 and 8. Only two other *Pycnophyes* species have paradorsal or middorsal setae on these particular segments: *P. rugosus* Zelinka, 1928 and *P. tubuliferus* (females on segment 2, 4, 6 and 8 and males on 6 and 8, see Adrianov 1989, Murakami *et al.* 2001). However, the first of these has a different distribution of lateroventral setae (segments 2-8 and 10) and number of apodemes (segments 9 and 10) (Zelinka 1928). The latter, *P. tubuliferus*, is one of three *Pycnophyes* species described from the area. *Pycnophyes chalgap* n. sp. and *P. tubuliferus* share several characters inclusive ventromedial sensory spot distribution, and distribution of setae in the lateroventral, ventrolateral and ventromedial positions (Adrianov 1989, Murakami *et al.* 2001). However, the two species can be differentiated by many other cuticular structures, including the long midsternal plate and other structures located on the dorsal side, such as the distribution of laterodorsal sensory spots and setae. *P. tubuliferus* has laterodorsal sensory spots on segments 3-9, whereas *Pycnophyes chalgap* n. sp. lacks them on segments 4, 6 and 8. *Pycnophyes chalgap* n. sp. has no laterodorsal setae on segment 6 while *P. tubuliferus* bears these (in fact the population of the Pacific coast of Japan has an additional pair of laterodorsal setae on this segment and in segments 2 and 4, see Murakami *et al.* 2001).

Regarding the presence of cuticular ornamentation as a net-like structure on the dorsal anterior margin of segment 1, it also appears in *P. dentatus*, *P. lageria*, *P. faveolus*, *P. furugelmi*, *P. oshoroensis*, *P. parasanjuanensis* and *P. sanjuanensis*. However none of them present the cuticular ornamentation with a semicircular profile: *P. lageria* has a profile that resembles candle wax drop (Sánchez *et al.* 2013), and *P. dentatus*, *P. faveolus*, *P. furugelmi*, *P. oshoroensis*, *P. parasanjuanensis* and *P. sanjuanensis* have a rectangular profile (see Zelinka 1928, Higgins 1961, Brown 1985, Adrianov and Higgins 1996, Adrianov and Malakhov 1999, Yamasaki *et al.* 2012).

Pycnophyes cristatus n. sp.
(Figs 9-11)

Type material. Holotype, adult male, collected on 11 November 2011 in the East Sea, off Uljin, locality MAP37 (Fig. 1C) 37°01′26″N, 129°30′55″E, from mud at 160 m depth; mounted in Fluoromount G®, deposited at the NHMD, under accession number ZMUC KIN-645. No females were available, hence no allotype is appointed. Paratype, adult male mounted for SEM, collected on the same date and at the same locality as the holotype, deposited at the NHMD, under accession number ZMUC KIN-646.

Diagnosis. *Pycnophyes* with middorsal processes on segments 1-10 and intracuticular atria on segments 1-9, but without any adjacent paradorsal seta. Middorsal processes of equal lengths until segment 6, turning progressively longer from this segment and towards the posterior ones. Middorsal process on segment 10 conspicuously long, extending beyond posterior margin of segment 11. Laterodorsal and ventromedial setae present only on segments 7-9.

Etymology. The species name, *cristatus*, is feminine, derived from Latin *crista*, crest, and refers to the conspicuous middorsal processes on segments 1-10.

Description. Mouth cone (Fig. 10I) and scalid arrangements by rings and sectors are apparently identical to the pattern described for *Pycnophyes chalgap* n. sp. (see Fig. 6). Only sector 6 could not be examined, so data from this sector are unavailable. The general scalid morphology also resembles that of *P. chalgap* sp. nov., except for the spinoscalids of rings 02 to 06 in *P. cristatus* n. sp., which possess a conspicuous long fringe near their attachment points (Fig. 10K).

Neck with four dorsal and two ventral placids (Figs 9A-B, 11C-D). All placids appear robust and hardly sclerotized, with concave surfaces. Dorsal placids rectangular, but with the medial ones slightly wider and with a notch in their middle regions (Fig. 11C). Ventral placids rectangular, extending from the lateral margin of the episternal plate to the midventral line (Fig. 11D). All placids join the anterior edge of the first trunk segment and extend anteriorly along the thin and flexible cuticle of the introvert.

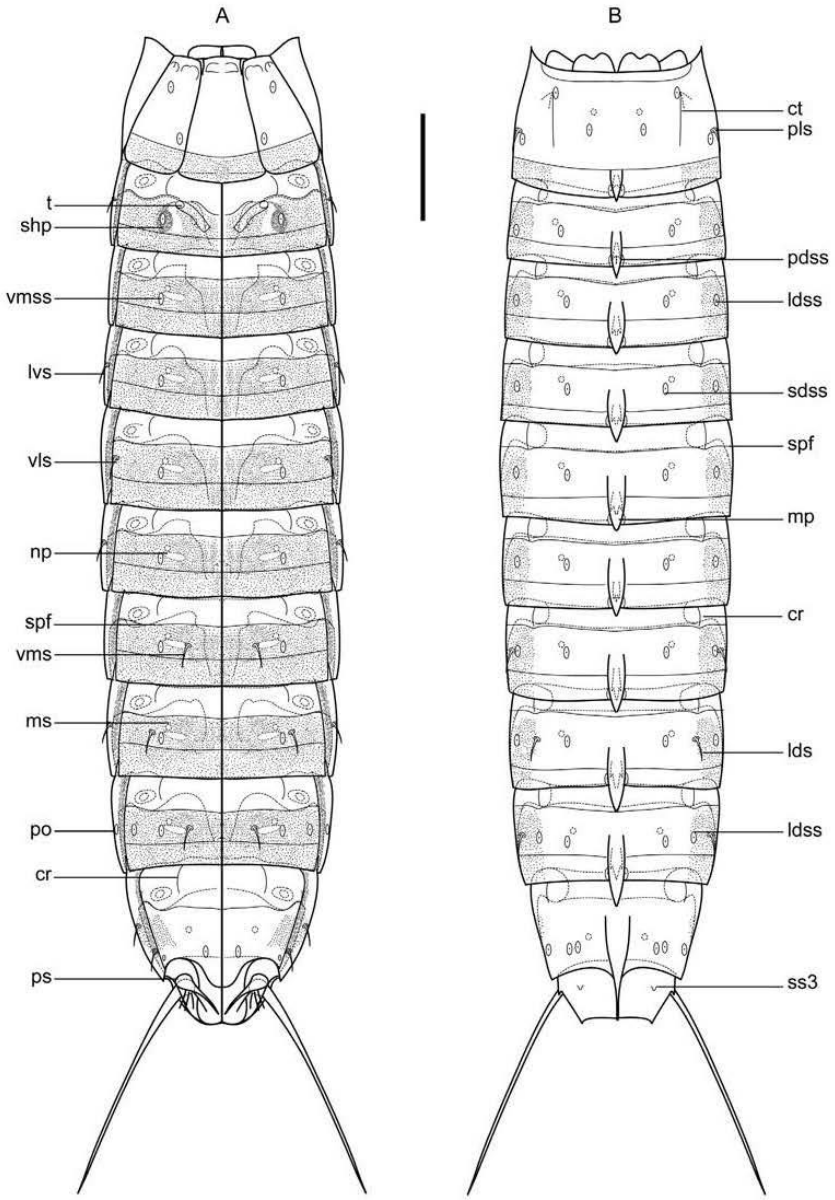

FIG. 9. – Line art illustrations of *Pycnophyes cristatus* n. sp. A, male, ventral view; B, male, dorsal view. Scale bar: 100 μm; *cr* cuticular ridge, *ct* cuticular thickening, *lds* laterodorsal seta, *ldss* laterodorsal sensory spot, *lvs* lateroventral seta, *mp* middorsal process, *ms* muscular scar, *np* naked patch, *pdss* paradorsal sensory spot, *pls* paralateral seta, *po* protonephridial opening, *ps* penile spine, *sdss* subdorsal sensory spot, *shp* short hair patch, *spf* secondary pectinate fringe, *ss3* sensory spot type 3, *t* tube, *vls* ventrolateral seta, *vms* ventromedial seta, *vmss* ventromedial sensory spot.

Trunk with 11 segments (Figs 9A-B, 10A, 11A-B), with first segment composed of one tergal and three ventral plates (Fig. 11D). Segments 2-11 with one tergal and two ventral sternal plates. Dimensions and measurements of the examined specimens are summarized in Table 5 and distribution of sensory spots and setae in Table 6. The segment width is nearly constant throughout the trunk, but reaches its maximum sternal width at segment 6 and tapers slightly from this segment. Segments 1 to 10 with middorsal processes, which always extend beyond the posterior margin of the segment (Figs 9B, 11B). These middorsal pro-

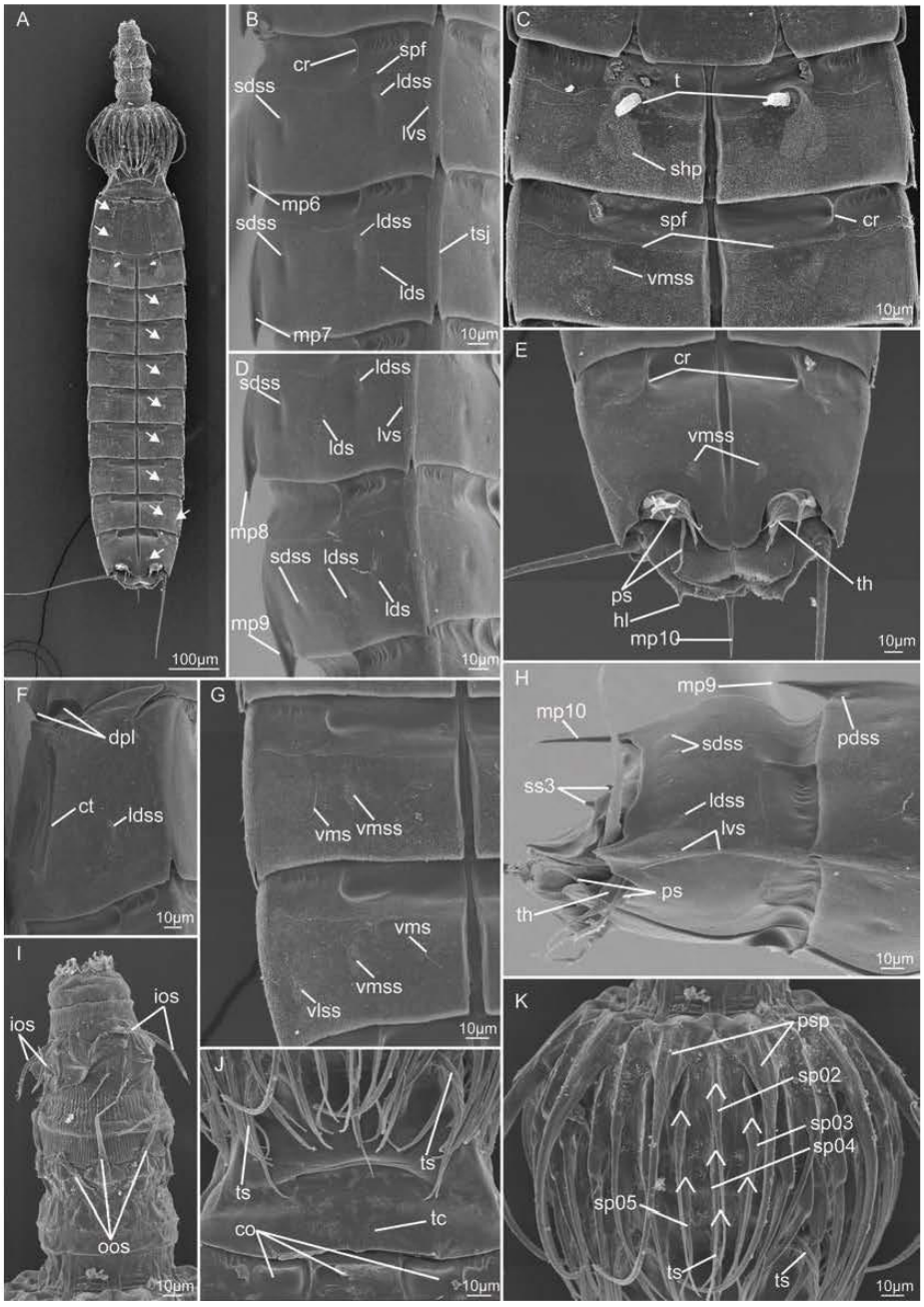

FIG. 10. – *Pycnophyes cristatus* n. sp. SEM photographs of male. A, ventral view; B, lateral view of segments 6-7; C, ventral view of segments 2-3; D, lateral view of segments 8-9; E, ventral view of segments 10-11; F, lateral view of segment 1; G, ventral view of segments 8-9; H, lateral view of segments 9-11; I, mouth cone; J, ventral view of segment 1 and the thin cuticle of the introvert; K, male, introvert sector 1; *co* cuticular ornamentation, *cr* cuticular ridge, *ct* cuticular thickening, *dpl* dorsal placid, *hl* horn-like extension, *ios* inner oral style, *lds* laterodorsal seta, *ldss* laterodorsal sensory spot, *lvs* lateroventral seta, *mp* middorsal process, *oos* outer oral style, *pdss* paradorsal sensory spot, *ps* penile spine, *psp* primary spinoscalid, *sdss* subdorsal sensory spot, *shp* short hairs patch, *sp* spinoscalid, *spf* secondary pectinate fringe, *ss3* sensory spot type 3, *t* tube, *tc* thin cuticle of the introvert, *th* tuft of hairs, *ts* trichoscalid, *tsj* tergosternal junction, *vlss* ventrolateral sensory spot, *vms* ventromedial seta, *vmss* ventromedial sensory spot. Sensory spots are marked with white arrows on A. Digits following labels refer to segment numbers except on K where they refer to the introvert ring numbers. Lambda symbols (Λ) mark attachment points of scalids.

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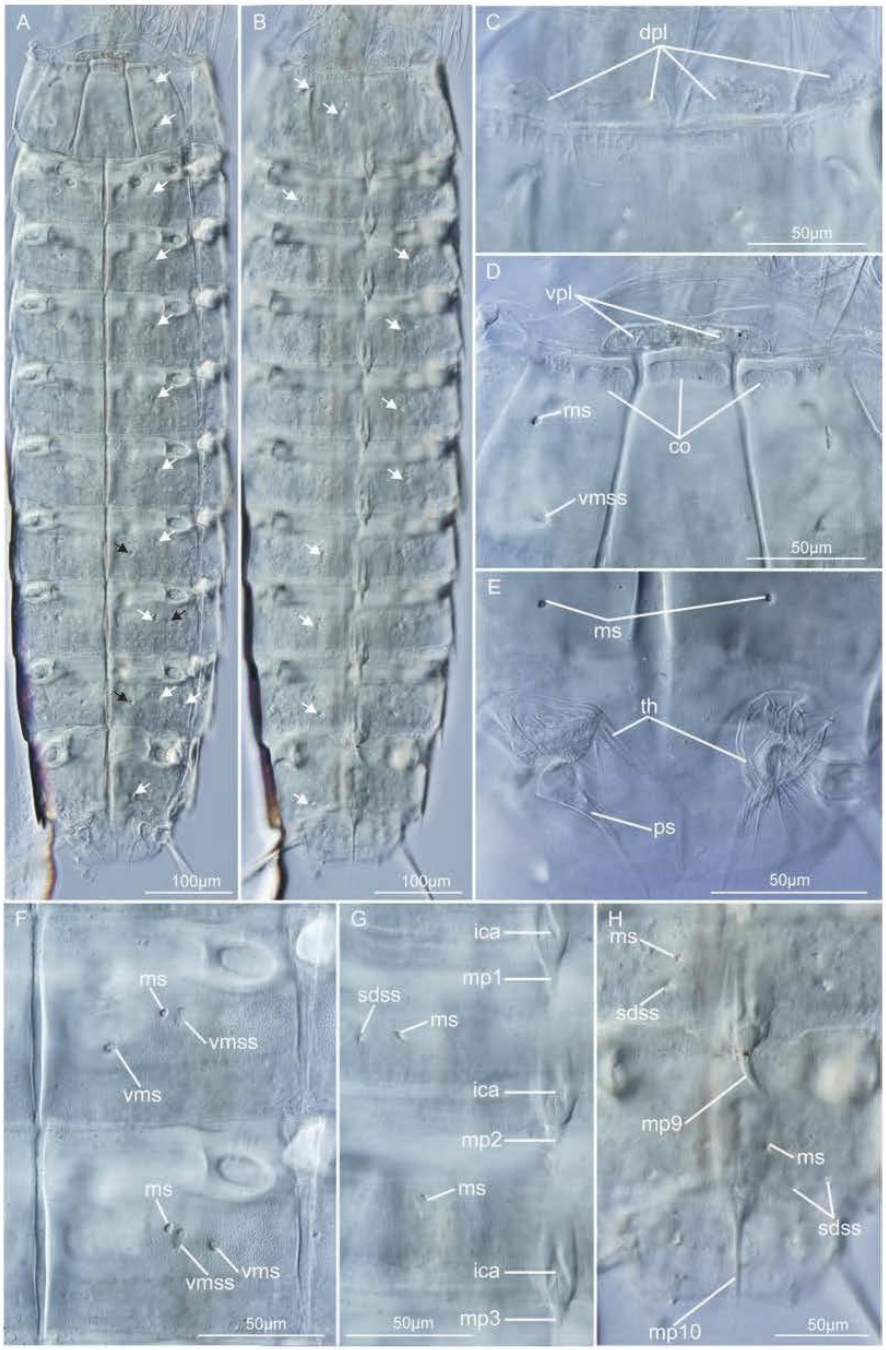

FIG. 11. – *Pycnophyes cristatus* n. sp. Differential interference contrast photographs of male. A, ventral view; B, dorsal view; C, dorsal view of neck and segment 1; D, ventral view of neck and segment 1; E, ventral view of segments 10-11; F, left sternal plates of segments 7-8; G, dorsal view of left half of segments 1-3; H, dorsal view of segments 9-11; *co* cuticular ornamentation, *dpl* dorsal placid, *ica* intracuticular atria of sensory spots, *mp* middorsal process, *ms* muscular scar, *ps* penile spine, *sdss* subdorsal sensory spot, *th* tuft of hairs, *vms* ventromedial seta, *vmss* ventromedial sensory spot, *vpl* ventral placid. Sensory spots are marked with white arrows. Setae are marked with black arrows. Digits following labels refer to segment numbers.

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TABLE 5. – Measurements (μm) of male holotype of *Pycnophyes cristatus* n. sp.; lts, lateral terminal spine; mps9-mps10, middorsal process length of segments 9-10; msw-6, maximum standard width of segment 6; sw, standard width; s1-s11, segment lengths of trunk segments 1-11; TL, trunk length.

Character	Length
TL	923
sw10	172
sw/TL	0.13%
msw-6	203
msw/TL	0.22%
lts/TL	0.24%
mps9/s9	0.34%
mps10/s10	0.40%
s1	117
s2	80
s3	81
s4	86
s5	86
s6	90
s7	91
s8	92
s9	91
s10	125
s11	44
lts11	220
mps9	31.6
mps10	49.7

TABLE 6. – Summary of location of setae and sensory spots in *Pycnophyes cristatus* n. sp. arranged by series. F, female condition of sexually dimorphic character; LD, laterodorsal; lts, lateral terminal spines; LV, lateroventral; m, male condition of sexually dimorphic character; ps, penile spines; PD, paradorsal; PL, paralateral; SD, subdorsal; se, setae; ss, sensory spots; ss3, sensory spot type 3; t, tubes; VL, ventrolateral; VM, ventromedial.

Segment	PD	SD	LD	PL	LV	VL	VM
1	ss	ss,ss	ss	se			ss,ss
2	ss	ss	ss		se		ss,t(m)
3	ss	ss	ss				ss
4	ss	ss	ss		se		ss
5	ss	ss	ss			se	ss
6	ss	ss	ss		se		ss
7	ss	ss	ss,se				se,ss
8	ss	ss	se,ss		se		ss,se
9	ss	ss	ss,se			ss	se,ss
10		ss,ss	ss		se,se	ss	ss
11		ss3(m)			lts	ps(2,m)	

cesses have a similar length until segment 6 and turn progressively longer from this point and towards the posterior segments, reaching the maximum size on segment 10 (Figs 10E, H, 11H). In addition, all segments with a pair of dorsoventral muscular scars and two pairs of cuticular ridges, one laterodorsal and one ventromedial (Fig. 9A-B). Apodemes not present. Peg and socket joints and pachycycli well developed and of similar sizes on segments 2-10 (Figs 9, 11A). Hairy tergosternal junctions with several long hairs present on segments 2-10.

Segment 1: Anterolateral margins of tergal plate project into horn-like extensions. Tergal anterior edge of the segment smooth, followed by a narrow area of cuticular ornamentation. Longitudinal cuticular thickenings present in subdorsal area, posterior to sensory spots (Fig. 10F). Posterior margin of dorsal plate with a middorsal process that surpasses the posterior margin of the segment, and one pair of paradorsal sensory spots associated with the intracuticular atria (Fig. 11G). Furthermore, tergal plate with one pair of muscular scars in the middle region of the segment, one pair of paralateral setae and three additional pairs of sensory spots, two in subdorsal and one in laterodorsal position (Figs 9B, 10F). Sensory spots on this and all following segments are rounded to elongate and belong to type 1 (except on segment 11). Ventral side with two episternal plates and one trapezoidal midsternal plate. Ventral anterior margin ornamented, followed by a contiguous concave area (Figs 10J, 11D). Each episternal plates with a muscular scar near its anterior margin and with two ventromedial sensory spots, one located on the anterior third of the plate and one on the posterior third (Fig. 10A). Short cuticular hairs present only on the posterior part, on both dorsal and ventral side. Pectinate fringes on dorsal and ventral sides inconspicuous, only visible with SEM.

Segment 2: Tergal plate with middorsal process, one pair of paradorsal sensory spots and associated intracuticular atria similar to those on segment 1 (Fig. 11G). Paired setae present in lateroventral positions, and sensory spots in subdorsal and laterodorsal positions. A pair of subdorsal muscular scars appears anterior to the subdorsal sensory spots on this and all following segments (Fig. 11G). One pair of laterodorsal cuticular ridges present. Sternal plates with one pair of ventromedial sensory spots and a pair of large ventromedial tubes located anterior to the sensory spots (Figs 10C, 11A). Cuticular ridges present in ventromedial positions. Short cuticular hairs present posterior to secondary pectinate fringe. Other kind of shorter cuticular hairs appear only on the sternal plates, forming a patch around the ventromedial sensory spots and the tubes. A mesial ventromedial smooth patch is located adjacent to this (Fig. 10C). Hairy tergosternal junctions with several long hairs present. Pectinate fringe on both dorsal and ventral sides similar to that on preceding segment. Secondary pectinate fringe on dorsal and ventral sides located in the anterior region of the segment, parallel to the anterior edge and surrounding the whole surface of the segment. Glandular cell outlets located anterolaterally on tergal and sternal plates, anterior to the secondary pectinate fringes. Pachycycli well developed, and peg and socket joints of normal size (Fig. 11A).

Segment 3: Tergal plate similar to that of segment 2, except for the absence of lateroventral setae (Fig. 11G). Sternal plates with one pair of ventromedial sensory spots. Cuticular hairs on sternal plates similar to those on the previous segment but without patches of shorter cuticular hairs. Paired smooth patches more elongate than those on segment 2. Other structures similar to those on preceding segment (Fig. 10C).

Segment 4: Tergal and sternal plates similar to those on segment 3, but with one pair of lateroventral setae.

Segment 5: Tergal and sternal plates similar to those

on segment 3, but with one pair of ventrolateral setae.

Segment 6: Tergal and sternal plates same as on segment 4 (Fig. 10B).

Segment 7: Tergal plate similar to that on segment 3, but with the middorsal process slightly more developed and with one pair of laterodorsal setae located lateral to the laterodorsal sensory spots (Fig. 10B). Sternal plates similar to those of segment 3, but also a pair of ventromedial setae, located close to the paraventral area (Fig. 11**A**, F). Otherwise similar to preceding segments.

Segment 8: Tergal plate similar to that of segment 4, but with the middorsal process more developed and with a pair of laterodorsal setae located mesial to the laterodorsal sensory spots (Fig. 10D). Sternal plates similar to those of segment 7, but with the ventromedial setae located more lateral than the sensory spots (Figs 10G, 11**A**, F). Otherwise similar to preceding segments.

Segment 9: Tergal plate with middorsal process more developed than on preceding segments (Figs 10H, 11H). Paradorsal sensory spots and their associated intracuticular atria similar to those on preceding segments. One pair of laterodorsal setae present in same position as the laterodorsal sensory spots on segment 8. Two pairs of sensory spots, one subdorsal and one laterodorsal, the latter in the same position as the laterodorsal setae on segment 8 (Fig. 10D). Protonephridial opening paralaterally, not sieve-like, surrounded by several short hairs. Sternal plates with a pair of ventromedial setae close to the paraventral position margin, and two pairs of sensory spots, one ventromedial in the same position as on previous segment, and one in ventrolateral position (Fig. 10G). Otherwise similar to preceding segments.

Segment 10: Tergal plate with middorsal process much more developed than on the previous segment, extending to or beyond terminal end of trunk. Paradorsal sensory spots and intracuticular atria not present (Figs 10H, 11B, H). Two pairs of lateroventral setae present. Three pairs of sensory spots located near the posterior margin of the segment, two in subdorsal and one in laterodorsal position (Figs 10H, 11H). Sternal plates with one pair of ventromedial and ventrolateral sensory spots, the latter being conspicuously smaller than the first one (Fig. 10E). Posterior margins of sternal plates with deep notches over the male genital pores (Figs 10E, 11E). Otherwise similar to preceding segments.

Segment 11: Middorsal process absent. With a pair of lateral terminal spines (Fig. 9**A**-B), and males with two pairs of penile spines and one pair of genital pores surrounded by a tuft of long hairs (Figs 10E, H, 11E). Tergal plate with two pairs of subdorsal type 3 sensory spots and one pair of horn-like cuticular extensions (Fig. 10E, H). Cuticular hairs and pectinate fringe similar to those on preceding segments.

Associated kinorhynch fauna. Pycnophyes cristatus n. sp. was found at locality MAP37 – a locality from a set of samples that are still under examination. Various still unidentified species of *Echinoderes* are present in the sample, together with a new of species of *Dracoderes* that is currently under description (pers. comm. M V. Sørensen).

Remarks. Pycnophyes cristatus n. sp. can be distinguished from most other species of *Pycnophyes* by the presence of its middorsal processes, which increase progressively in length from segment 7. Especially the middorsal process of segment 10 is very conspicuous and extends well beyond the posterior margin of the segment. Six other species bear middorsal processes that increase in length towards the posterior segments. These include *P. carinatus*, *P. arctous* Adrianov, 1999; *P. chilensis* Lang, 1953; *P. chukchiensis* Higgins, 1991; *P. furugelmi* and *P. odhneri* Lang, 1949 (see Zelinka 1928, Lang 1949, 1953, Higgins 1991, Adrianov and Malakhov 1999). However, only three of these species possess a middorsal process of segment 10 extending well beyond the posterior margin of the segment: *P. arctous*, *P. chukchiensis* and *P. furugelmi*. *P. chukchiensis* and *P. furugelmi* both possess ventromedial setae on segments 3-6 (Higgins 1991, Adrianov and Malakhov 1999), whereas *Pycnophyes cristatus* n. sp. has no ventromedial setae on these segments. Moreover, these species differ from *P. cristatus* n. sp. by their distribution of sensory spots and laterodorsal and lateroventral setae: *P. chukchiensis* has ventromedial sensory spots only, specifically on segments 1-10, laterodorsal setae on segments 2-9, and lateroventral ones on segments 2-10 (Higgins 1991); and *P. furugelmi* has ventromedial sensory spots only, specifically on segments 3-8, laterodorsal setae on segments 4-8 in males and the females with ventromedial sensory spots on segments 3-6 and 9, and laterodorsal setae on segments 2, 3, 4, 6, 8 (Adrianov and Malakhov 1999). Though *P. arctous* lacks ventromedial setae on segments 3-6, like the new species, it has no ventromedial setae on segments 7-9, as found in *Pycnophyes cristatus* n. sp. (see Adrianov and Malakhov 1999). Furthermore, *P. arctous* differs from *Pycnophyes cristatus* n. sp. by its appearance of middorsal processes of segments 1-7, which are nearly obtuse, whereas all the middorsal processes of *Pycnophyes cristatus* n. sp. are pointed.

Pycnophyes smaug sp. nov.
(Figs 12-15)

Type material. Holotype, adult female, collected on 11 November 2011 in the East Sea, off Uljin, locality MAP37 (Fig. 1C) 37°01′26″N, 129°30′55″E, from mud at 160 m depth; mounted in Fluoromount G®, deposited at the NHMD, under accession number ZMUC KIN-647. Allotype, adult male, collected on same date and at the same locality as the holotype, mounted in Fluoromount G®, deposited at NHMD under accession number ZMUC KIN-648. Four additional specimens, one male and three females, collected on the same date and at the same locality as the holotype, mounted for SEM, are stored in the last author's collection and will later be included in the NHMD general collection.

Diagnosis. *Pycnophyes* with middorsal elevations and intracuticular atria on segments 1-9, but without paradorsal setae flanking the middorsal elevations. Ventral anterior margin of segment 1 bulged and ornamented. Tergal anterior margin of segment 1 denticulated and ornamented. Trunk segments generally with few setae, present only as laterodorsal setae on segments 2 and 9, lateroventral setae on segments 2, 4, 6, 8 and 10, ventrolateral setae on segment 5 and ventromedial setae on segment 9 (females also on segment 2). Type 2 sensory spots on segments 1-10.

Etymology. Like other recently described kinorhynchs, the species is named after a dragon. The species name *smaug*, refers to the dragon Smaug, the greatest and most powerful in the later part of the Third Age in the books of J.R.R. Tolkien.

Description. Introvert and mouth cone: mouth cone (Fig. 14N) and spinoscalid arrangements by rings and sectors (Figs 13, 14O) are identical to the pattern described for *Pycnophyes chalgap* n. sp., except for sector 6, which shows the same scalid pattern as other even numbered sectors (unlike *Pycnophyes chalgap* n. sp., which has a small number of spinoscalids in this sector). The general spinoscalid morphology also resembles that in *Pycnophyes cristatus* n. sp. See Figure 13 for a complete summary of oral style and scalid and placid locations.

Neck with four dorsal and two ventral placids, appearing robust and hardly sclerotized, with concave surfaces. The two medial dorsal placids are wider, with a rectangular profile and a notch in their middle regions, whereas the two lateral ones are narrower with a square profile without a notch (Figs 12B, 15C). Ventral placids rectangular, extending from the lateral margin of the episternal plate to the midventral line (Fig. 12A). All placids articulate with the first trunk segment and extent anteriorly along the thin and flexible cuticle of the introvert.

Trunk composed of 11 segments (Figs 12A-B, 14A-C, 15A-B), with first segment composed of one tergal and three sternal plates (Fig. 14L). Segments 2-11 with one tergal and two sternal plates. All dimensions and measurements of the examined specimens are summarized in Table 7 and distribution of sensory spots and setae in Table 8. The segment width is almost constant throughout the trunk, but maximum sternal width is reached at segment 6, and the segments turn slightly narrower from this point. Segments 1-9 with paradorsal intracuticular atria and middorsal elevations that never extend beyond the posterior margin of the segment (Fig. 14A). Segments 3-9 with at least two pairs of small oblique dorsoventral muscular scars. One pair of laterodorsal cuticular ridges is present on segments 2-10 and two pairs of subdorsal ones on segments 8-10. One pair of ventromedial cuticular ridges present on segments 2-8 and two pairs on segments 9-10 (Fig. 12A-B). Pachycycli of similar size, and well developed from segments 2-10. Peg and socket joints well developed on segments 2-8 and less developed on segments 9 and 10. Hairy tergosternal junction on segments 2-10 with three kinds of short hairs (e.g. Fig. 14F).

Segment 1: Anterolateral margins of tergal plate project into horn-like extensions. Tergal anterior margin finely denticulated and with a narrow reticulated area. Posterior margin of dorsal plate with a middorsal elevation (never surpassing the posterior margin of the segment), flanked by a pair of paradorsal sensory spots with associated intracuticular atria only visible with DIC (Fig. 15G). Tergal plate with three additional pairs of sensory spots, one in subdorsal and two in laterodorsal positions (Figs 12B, 14A-B, 15C). Sensory spots on this and all the following segments belong to type 2 (except on segment 11), being rounded to elongate and consisting of two pores surrounded by several small cuticular papillae (e.g. Fig. 14G). One pore is located centrally in the sensory area and the other, more elevated one, in the anterior region. Small oblique muscular scars present in subdorsal position. Ventral side with two episternal plates and one trapezoidal midsternal plate; posterior margin of midsternal plate with a small, pointed midventral process (Figs 12A, 14L). Ventral anterior margin with a depressed area. Each episternal plate with one ventromedial sensory spot near the anterior margins (Fig. 15A) and with a muscular scar in the middle region. Tergosternal junction inconspicuous. Pectinate fringes on both dorsal and ventral sides inconspicuous, only visible with DIC.

Segment 2: Tergal plate with middorsal elevation; associated paradorsal sensory spots and intracuticular atria similar to those on segment 1 (Figs 14D, 15G). A pair of laterodorsal and lateroventral setae present. Four additional pairs of sensory spots present as one subdorsal, two laterodorsal and one lateroventral pair, the latter being smaller than the other ones (Fig. 14D and similar to Fig. 14E). Laterodorsal pair located most mesially is more rounded and smaller than the other laterodorsal and subdorsal pairs. The laterodorsal pair of sensory spots located most laterally appears at the same position as the laterodorsal cuticular ridges (Fig. 14D). One pair of oblique subdorsal muscular scars present. Patch of scale-like cuticular hairs from the tergosternal junctions to the position of the laterodorsal cuticular ridges. Sternal plates with one pair of ventromedial sensory spots (Fig. 15A), located in the same position as the ventromedial cuticular ridges. Males with pair of big tubes located in the ventromedial position (Fig. 12C); females with a pair of ventromedial setae (Figs 12A, 15A). One pair of ventromedial muscular scars. Patch of scale-like cuticular hairs present along the sternal plates from their lateral margins to the position of the cuticular ridges (similar to Fig. 14H). Hairy tergosternal junctions divided into three portions, each one with different kinds of cuticular hairs (Fig. 14F): the anteriormost with several short and thin hairs only joined to the trunk by the base; the next portion with wider cuticular hairs joined by their whole length with

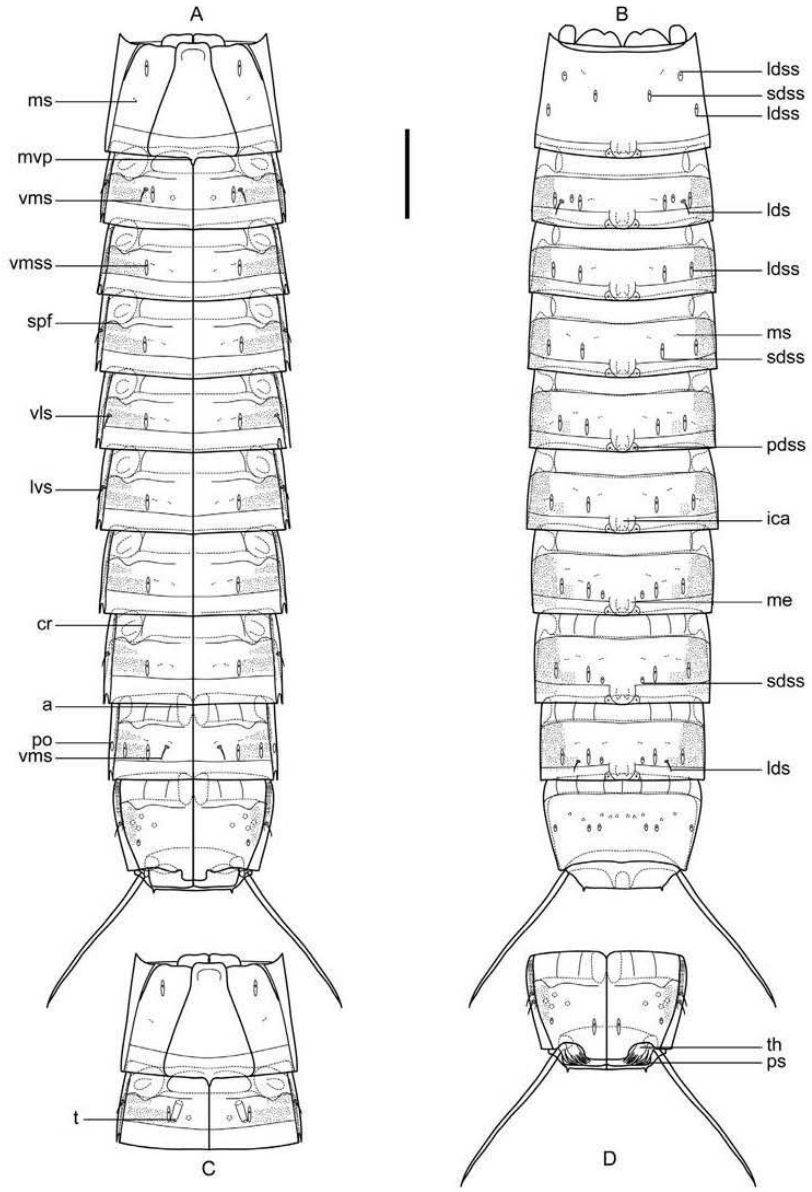

FIG. 12. – Line art illustrations of *Pycnophyes smaug* n. sp. A, female, ventral view; B, female, dorsal view; C, male, segments 1-2, ventral view; D, male, segments 10-11, ventral view. Scale bar: 100 µm; *a* apodeme, *cr* cuticular ridge, *ica* intracuticular atria of sensory spot, *lds* laterodorsal seta, *ldss* laterodorsal sensory spot, *lvs* lateroventral seta, *me* middorsal elevation, *ms* muscular scar, *mvp* midventral process, *pdss* paradorsal sensory spot, *po* protonephridial opening, *ps* penile spine, *sdss* subdorsal sensory spot, *spf* secondary pectinate fringe, *t* tube, *th* tuft of hairs, *vls* ventrolateral seta, *vms* ventromedial seta, *vmss* ventromedial sensory spot.

the surface and only with the free tips; and the posteriormost portion with longer and wider cuticular hairs joined along the surface by their whole length, without the free tips. Secondary pectinate fringe on dorsal and ventral sides located in the anterior region of the segment, parallel to the anterior edge and surrounding the whole segment, except in the paraventral region. Glandular cell outlets present on tergal and sternal plates on this and all following segments, located anterolaterally, anterior to the secondary pectinate fringes (Fig. 14D). Pachycycli well developed, with a normal size of the peg and socket joints.

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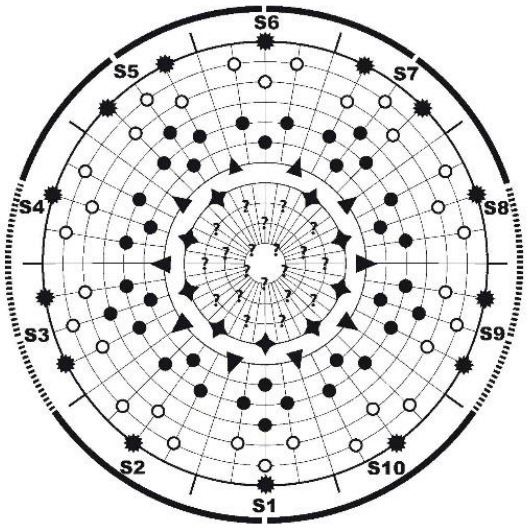

Scalid and style arrangement

Ring/Sector		1	2	3	4	5	6	7	8	9	10	Total
00 oos	✦	1	1	1	1	1	0	1	1	1	1	9
01	▼	1	1	1	1	1	1	1	1	1		10
02	●	1	1	1	1	1	1	1	1	1	1	10
03	●	2	2	2	2	2	2	2	2	2	2	20
04	●	1	0	1	0	1	0	1	0	1	0	5
05	○	2	1	2	1	2	1	2	1	2	1	15
06	○	1	2	1	2	1	2	1	2	1	2	15
07 tr	✸	1	1	2	1	2	1	2	1	2	1	14
Total scalids		8	7	9	7	9	7	9	7	9	7	89

FIG. 13. – Diagram of mouth cone, introvert and placids in *Pycnophyes smaug* n. sp. with the distribution and type of scalids by ring and sector. Dashed lines mark flexible cuticular lateral areas. "Double diamonds" are marked in the table with double lines. *oos* outer oral style, *tr* trichoscalid, *s1–s10* sector number.

Segment 3: Tergal plate with middorsal elevation; associated paradorsal sensory spots and intracuticular atria similar to those on preceding segments (Fig. 15G). Three pairs of sensory spots present in subdorsal, laterodorsal and lateroventral positions (Fig. 14D). The first two are large and elongated, whereas the lateroventral ones are smaller and wider. Two pairs of oblique muscular scars present in subdorsal and laterodorsal positions (Fig. 15G). Sternal plates with one pair of ventromedial sensory spots in the same position as on preceding segment (Figs 14C, 15F). Two pairs of ventromedial muscular scars (Fig. 15F). Tergosternal junctions, glandular cell outlets, pachycycli, peg and socket joints, pectinate fringe and secondary pectinate fringe as on preceding segment.

Segment 4: Tergal and sternal plates similar to those on segment 3 but with a pair of lateroventral setae (Figs 14E, G, 15F).

Segment 5: Tergal and sternal plate similar to those on segment 3, but with a pair of ventrolateral setae. The patches of scale-like cuticular hairs on the tergal plate extend towards the subdorsal position. A couple of scale-like cuticular hairs appear mesially on the sternal plates. Otherwise similar to preceding segment.

Segment 6: Tergal and sternal plates similar to those on segment 4. Segment otherwise as preceding segment.

Segment 7: Tergal and sternal plates similar to those on segment 3, but with an additional pair of subdorsal sensory spots present, close to the middorsal elevation (Fig. 14B). The additional sensory spots are rounded, opposed to the other elongated ones. One pair of apodemes present near anterior margin of segment, only visible in males. Patches of cuticular hairs on both dorsal and ventral side similar to those on segment 5. Otherwise similar to preceding segment.

Segment 8: Tergal plate similar to segment 7 but with a pair of lateroventral setae and two additional pairs of cuticular ridges in subdorsal positions. Sternal plates same as on segment 3 (Fig. 14H, M). One pair of apodemes present near anterior margin of segment, only visible in males. Patches of cuticular hairs on both dorsal and ventral side similar to those on segment 5. Otherwise similar to preceding segment.

Segment 9: Tergal plate similar to segment 8, except for the setae that appear in laterodorsal and not lateroventral positions (Fig. 14B). Protonephridial opening, not sieve-like, present in paralateral position

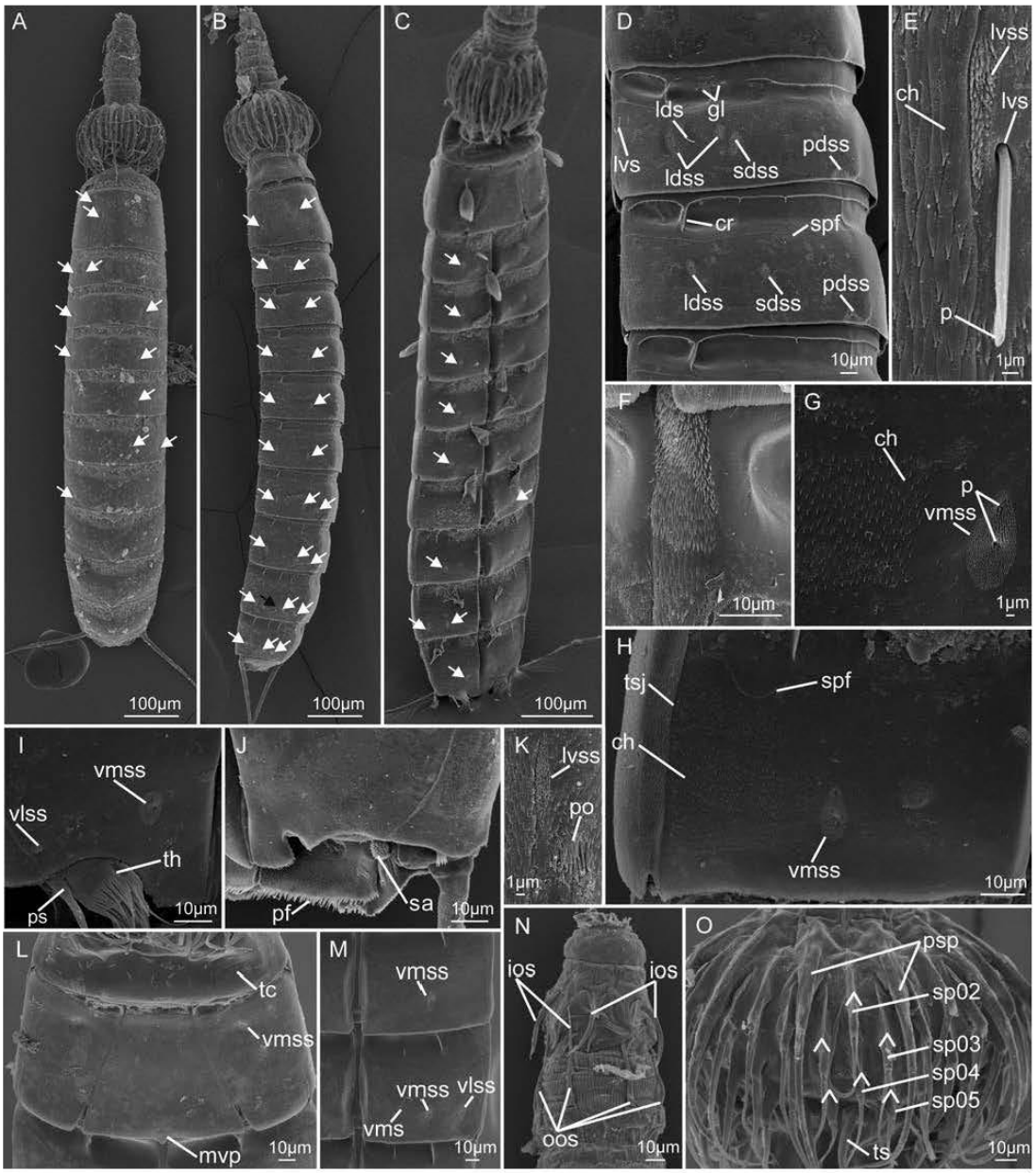

FIG. 14. – *Pycnophyes smaug* n. sp. SEM photographs. A, female, dorsal view; B, female, lateral view; C, male, ventral view; D, female, lateral view of segments 2-3; E, female, tergosternal area of segment 4; F, female, segment 2, tergosternal junction with three kinds of hairs; G, male, ventromedial position of segment 4; H, male, right sternal plate of segment 8; I, male, left sternal plate of segment 10; J, female, left sternal plate of segments 10-11; K, female, lateral view of segment 9, protonephridial opening; L, female, ventral view of segment 1 and the thin cuticle of the introvert; M, female, left sternal plates of segments 8-9; N, female, mouth cone; O, female, introvert sector 8; *ch* cuticular hairs, *cr* cuticular ridge, *gl* glandular cell outlets, *ios* inner oral style, *lds* laterodorsal seta, *ldss* laterodorsal sensory spot, *lvs* lateroventral seta, *lvss* lateroventral sensory spot, *mvp* midventral process, *oos* outer oral style, *p* pore, *pdss* paradorsal sensory spot, *pf* pectinate fringe, *po* protonephridial opening, *ps* penile spine, *psp* primary spinoscalid, *sa* sensory area, *sdss* subdorsal sensory spot, *sp* spinoscalid, *spf* secondary pectinate fringe, *tc* thin cuticle of the introvert, *th* tuft of hairs, *ts* trichoscalid, *tsj* tergosternal junction, *vlss* ventrolateral sensory spot, *vms* ventromedial seta, *vmss* ventromedial sensory spot. Sensory spots are marked with white arrows and setae with black arrows on A-C. Digits following labels on O refer to the introvert ring numbers. Lambda symbols (Λ) mark attachment points of scalids.

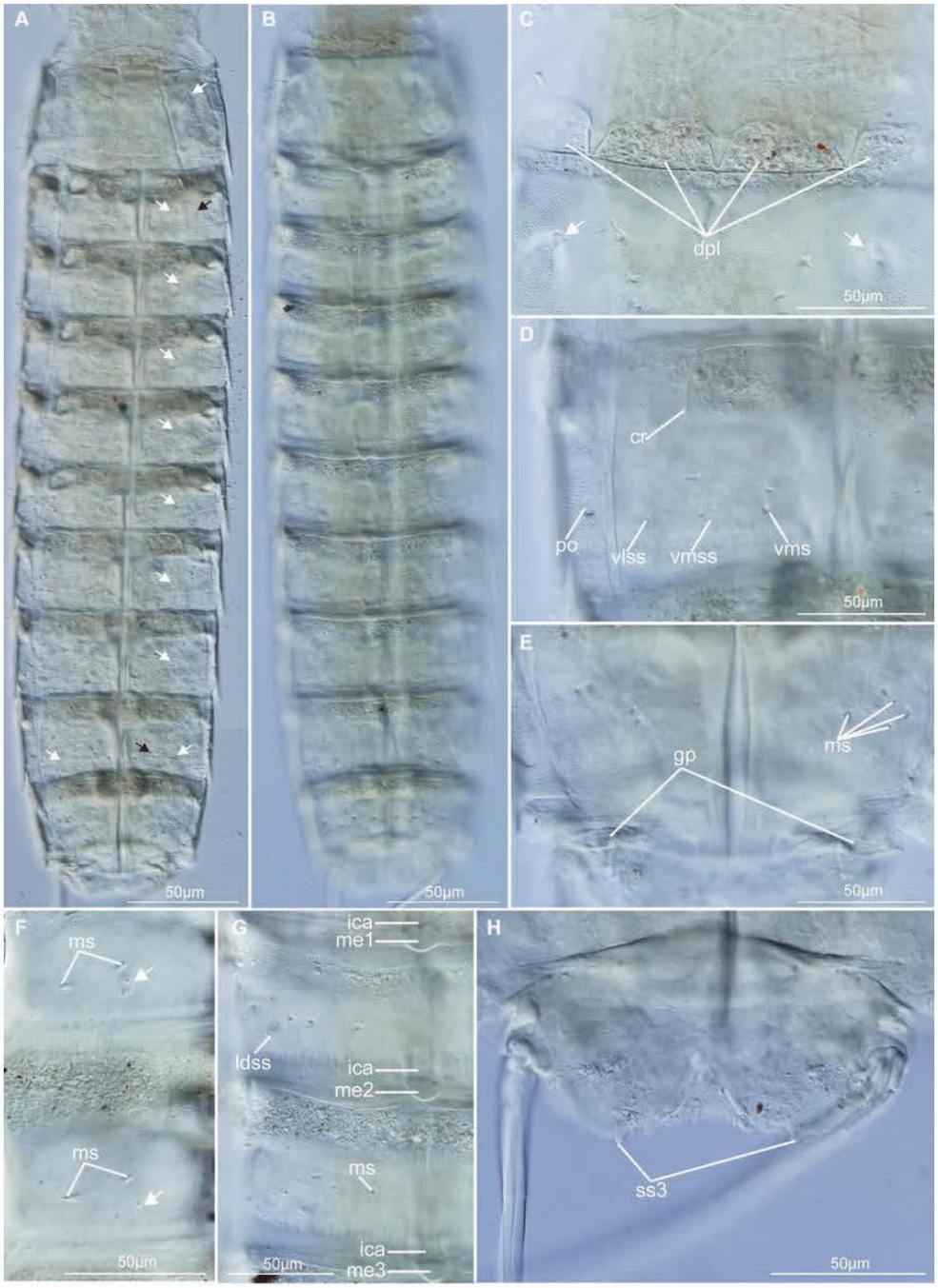

FIG. 15. – *Pycnophyes smaug* n. sp. Differential interference contrast photographs. A, female, ventral view; B, female, dorsal view; C, female, dorsal view of neck and segment 1; D, female, left sternal plates of segment 9; E, female, ventral view of segment 10; F, male, right sternal plates of segments 3-4; G, female, dorsal view of left half of segments 1-3; H, female, dorsal view of segment 11; *cr* cuticular ridge, *dpl* dorsal placid, *gp* gonopore, *ica* intracuticular atria of sensory spots, *ldss* laterodorsal sensory spot, *me* middorsal elevation, *ms* muscular scar, *po* protonephridial opening, *ss3* sensory spot type 3, *vlss* ventrolateral sensory spot, *vms* ventromedial seta, *vmss* ventromedial sensory spot. Sensory spots are marked with white arrows. Setae are marked with black arrows. Digits following labels refer to segment numbers.

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TABLE 7. – Measurements of female holotype and male allotype of *Pycnophyes smaug* n. sp.; lts, lateral terminal spine; msw-6, maximum standard width of segment 6; sw, standard width; s1-s11, segment lengths of trunk segments 1-11; TL, trunk length.

Character	Length	
	holotype ♀	allotype ♂
TL	960	897
sw10	169	179
sw/TL	0.18%	0.20%
msw-6	200	201
msw/TL	0.21%	0.22%
lts/TL	0.18%	0.22%
s1	127	126
s2	88	86
s3	93	90
s4	89	92
s5	89	95
s6	96	101
s7	96	104
s8	97	104
s9	101	106
s10	124	135
s11	70	55
lts11	170	201

(Fig. 15D); pore surrounded by few short hairs (Fig. 14K). Sternal plates similar to those on previous segments but with one additional pair of ventromedial cuticular ridges, ventromedial setae and ventrolateral sensory spots (Figs 14M, 15D). One pair of apodemes present near anterior margin of segment, visible in both sexes (Fig. 12A). Patches of cuticular hairs on both dorsal and ventral side similar to those on segment 5. Peg and socket joints inconspicuous. Otherwise similar to preceding segment.

Segment 10: Middorsal structure (middorsal process or elevation) or intracuticular atria absent. Tergal plate with two pairs of lateroventral setae in males, and only one pair in females. Three pairs of sensory spots present, two in subdorsal and one in laterodorsal positions (Fig. 14B). Two pairs of cuticular ridges present in subdorsal position, and one in laterodorsal. Males with one pair of ventromedial and ventrolateral sensory spots, the latter smaller than the other; and posterior margins of sternal plates with deep notches over the genital pores (Figs 12D, 14I). Females only with one pair of small ventrolateral sensory spots. Posterior sternal segment margin in females straighter than in males, with small but deep notches near the paraventral positions (Fig. 14J). Both sexes with two pairs of ventromedial cuticular ridges. Hairy tergosternal joints are not present along the whole lateral margins. One pair of apodemes present near anterior margin (Fig. 12A-D). Peg and socket joints very indistinct. Patches of cuticular hairs on both dorsal and ventral side, similar to those on segment 4. Several small and rounded muscular scars appear on the dorsal and ventral sides (Fig. 15B, E). Otherwise similar to preceding segment.

Segment 11: With a pair of lateral terminal spines (Fig. 12A-B). Tergal plate with one pair of small, hornlike cuticular extensions. Males with two pairs of penile spines and a pair of genital pores surrounded by a tuft of long hairs (Figs 12D, 14I). Females with one pair of type 3 sensory spots (Fig. 15H) and a spherical sensory area located in the ventral part of the tergal plate, so these structures are visible in ventral view (Fig. 14J).

TABLE 8. – Summary of location of setae and sensory spots in *Pycnophyes smaug* n. sp. arranged by series. f, female condition of sexually dimorphic character; LD, laterodorsal; lts, lateral terminal spines; LV, lateroventral; m, male condition of sexually dimorphic character; ps, penile spines; PD, paradorsal; SD, subdorsal; se, setae; ss, sensory spots; ss3, sensory spot type 3; t, tubes; VL, ventrolateral; VM, ventromedial.

Segment	PD	SD	LD	LV	VL	VM
1	ss	ss	ss,ss			ss
2	ss	ss	ss,se,ss	se,ss		ss,se(f),t(m)
3	ss	ss	ss	ss		ss
4	ss	ss	ss	se,ss		ss
5	ss	ss	ss	ss	se	ss
6	ss	ss	ss	se,ss		ss
7	ss	ss,ss	ss	ss		ss
8	ss	ss,ss	ss	se,ss		ss
9	ss	ss,ss	se,ss	ss	ss	ss,se
10		ss,ss	ss	se,se(m)	ss	ss(m)
11		ss3(m)		lts	ps(2,m)	

Associated kinorhynch fauna. See under description of *Pycnophyes cristatus* n. sp.

Remarks. Pycnophyes smaug n. sp. has, apart from its lateroventral setae, only setae in laterodorsal positions on segments 2 and 9, and in ventromedial position on segment 9. Several other species share the absence of middorsal or paradorsal setae on all segments, but only seven of them have such a scarcity of setae on the whole dorsal side as found in *Pycnophyes smaug* n. sp. These include *P. argentinensis* Martorelli and Higgins, 2004; *P. arctous, P. canadensis* Higgins and Korczynski, 1989; *P. chilensis*, *P. maximus* Reimer, 1963; *P. odhneri* and *P. ponticus* (Reinhard, 1881) (see Reinhard 1881, Lang 1949, 1953, Reimer 1963, Higgins and Korczynski 1989, Adrianov and Malakhov 1999, Martorelli and Higgins 2004).

However, *P. chilensis* and *P. maximus* do not have any kind of setae at all (see Lang 1953, Reimer 1963), *P. canadensis* has subdorsal setae on segment 1 (see Higgins and Korczynski 1989), whereas the remaining species only show setae in lateroventral position on the tergal plates: *P. arctous* on segment 2 (in females only), *P. argentinensis* on segment 6, *P. odhneri* on segments 2, 4, 6, 8 and 10, and *P. ponticus* on segments 2-10 (see Reinhard 1881, Lang 1949, Adrianov and Malakhov 1999, Martorelli and Higgins 2004) and hence not in laterodorsal position of segments 2 and 9 as found in *Pycnophyes smaug* n. sp. Moreover, *P. odhneri* differs from *Pycnophyes smaug* n. sp. in the presence of ventromedial setae on all segments (Lang 1949), whereas *Pycnophyes smaug* n. sp. has ventromedial setae on segment 9 only. *P. ponticus* has ventromedial setae on a single segment only, and it appears on segment 4 (Reinhard 1881), and not segment 9 as in *Pycnophyes smaug* n. sp.

Pycnophyes smaug n. sp. can also be distinguished from the above species by other characters. The new species has a midventral process on the posterior margin of midsternal plate, whereas none of the seven species discussed has this structure. Furthermore, *P. arctous*, *P. chilensis* and *P. odhneri* have long middorsal processes, which clearly extend beyond the posterior margin of segments 9-10 in the former species (Adrianov and Malakhov 1999) and of segment 9 in the other two species (Lang 1949, 1953). Information on sensory spot distribution is unfortunately not available for any of the species discussed here.

DISCUSSION

The description of four new species brings the total number of valid kinorhynch species around the Korean Peninsula up to 26. Since additional new species are currently under description (M. V. Sørensen, pers. comm.), the Korean kinorhynch fauna now can be considered among the most well-examined in the world. Only the Iberian Peninsula has been subject to similar intensive studies, and from this region we also know close to 30 species (Sánchez *et al.* 2012). Similar studies of other, comparable regions would be interesting to determine whether Spain and Korea hold a particularly high kinorhynch diversity, or whether the high species numbers are due to the great sampling effort made in the area, which means that similar high diversities in other places could be expected at these latitudes.

With eight known *Pycnophyes* species (*P. furugelmi*, *P. oshoroensis*, *P. schornikovi* and *P. tubuliferus*, plus the four described herein), in the Korean-Western Japanese region, the genus's diversity is beginning to reach levels known from other comparable regions. For instance, a total of nine *Pycnophyes* species are now known from the waters around the Iberian Peninsula in Europe (Sánchez *et al.* 2012). The two regions are comparable in size, situated in subtropical regions and relatively well-sampled, so this may give us a hint about the diversity we can expect to find in such areas. For comparison, 6 species are known from the slightly colder temperate Scandinavian inland waters, including the Baltic Sea and Kattegat (Higgins 1983), and only two from the arctic West Greenland, though especially the Disko Island and Disko Bay area has been sampled very intensively by Higgins and Kristensen (1988), and through numerous student field courses since the mid-1980s. On the other hand, 12 species are known from the warmer Mediterranean Sea. This could be a weak indication showing that diversity of the genus increases from the colder Polar Regions towards the equator. Our knowledge on global kinorhynch distribution in general is of course far too limited to draw this conclusion yet, but the limited amount of data that is currently available points in this direction. Comparable studies from tropical regions are not yet available because the few studies that have been carried out closer to the equator focus on much smaller areas, but it would be desirable to study whether diversity, as expected, is higher in such areas.

With the addition of the four new species described in the present contribution, and two additional ones described recently (Sánchez *et al.* 2013) the number of valid *Pycnophyes* species based on descriptions of adult specimens will reach 53, and hence strengthen the genus' position as the second most diverse after *Echinoderes*. We still need to understand why these two particular genera stand out as the most successful in terms of speciation, but at least the present study has confirmed that *Pycnophyes* is among the most diverse.

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Deep-sea Kinorhyncha: two new species from the Guinea Basin, with evaluation of an unusual male feature

Nuria Sánchez, Fernando Pardos, Martin V. Sørensen

RESUMEN: **Kinorrincos de las profundidas: dos nuevas especies de la cuenca de Guinea, con evaluación de un carácter masculino poco común.** —Se describen dos nuevas especies de kinorrincos homalorrágidos de las aguas profundas de la cuenca de Guinea. *Pycnophyes nubilis* sp. nov. es fácilmente reconocible por la presencia de sedas paradorsales en el segment 1; salientes mediodorsales en los segmentos 1-10, aumentando en tamaño hacia los segmentos posteriors; y un saliente mediodorsal en el segmento 10 que se extiende sobre el último segmento. *Pycnophyes farinellii* sp. nov. se distingue por la presencia de elevaciones mediodorsales en los segmentos 2-9 y sedas laterodorsales y ventromediales únicamente en los segmentos 2 y 9. Además, los machos de la última especie carecen de tubos en el segmento 2—un carácter muy poco común entre las especies del género. La relevancia sistemática de dicho carácter es discutida en detalle.

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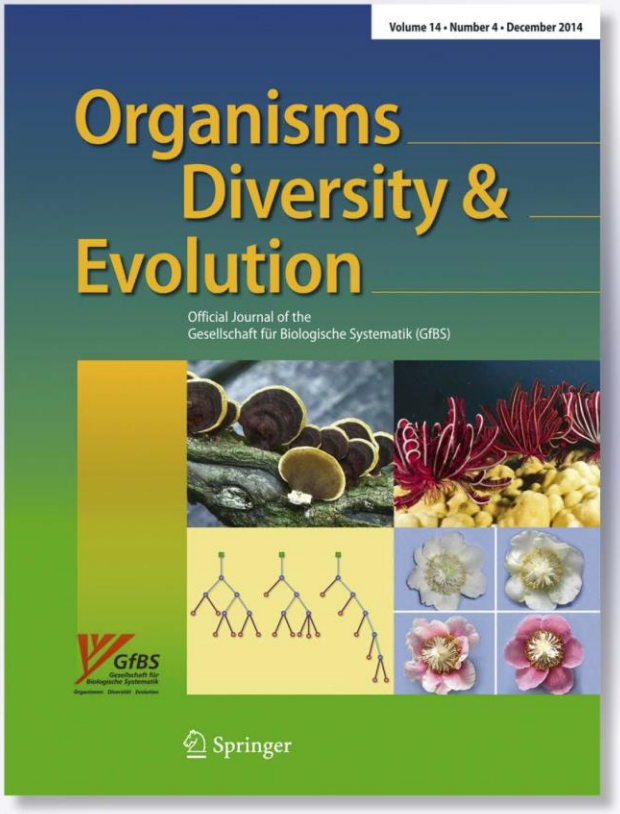

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ORIGINAL ARTICLE

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Deep-sea Kinorhyncha: two new species from the Guinea Basin, with evaluation of an unusual male feature

Nuria Sánchez · Fernando Pardos · Martin V. Sørensen

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Abstract Two new species of homalorhagid kinorhynchs from the deep-sea Guinea Basin are described. *Pycnophyes nubilis* sp. nov. is easily recognized by the presence of paradorsal setae on segment 1; middorsal processes on segments 1–10, progressively increasing in length towards the posterior segments; and middorsal process on segment 10 extending over the last trunk segment. *Pycnophyes farinellii* sp. nov. is distinguished by middorsal elevations on segments 2–9 and laterodorsal and ventromedial setae on segments 2 and 9 only. Moreover, males of the latter species lack ventromedial tubes on segment 2—a very uncommon feature among the genus. The systematic significance of this character is discussed in detail.

Keywords Meiofauna · Kinorhynch · Pycnophyes · Taxonomy · Diva2

Introduction

Kinorhyncha is a phylum of marine meiobenthic invertebrates comprising over 200 described species (Herranz et al. 2014; Sánchez et al. 2014a, b; Sørensen 2013). Kinorhynchs may be found at different depths, from deep to shallow waters, or even in intertidal sediments, but they have mostly been recorded at relatively shallow waters (Adrianov and Malakhov 1999; Sørensen and Pardos 2008; Neuhaus 2013), reflecting just sampling strategies rather than true distribution. The deepest kinorhynch ever recorded was found in the abyssal zone, at 7,800 m depth in the Atacama Trench (Danovaro et al. 2002). However, it was not identified to even class level. The species from the deepest locality identified to species level is *Mixtophyes abyssalis* Sánchez et al. 2014, collected during the R/V Meteor DIVA2 Cruise and recently described. It was recorded at 5,136 to 5,174 m depth in the Guinea Basin. The discovery also prompted the description of the new homalorhagid genus *Mixtophyes*, the latest homalorhagid genus described (Sánchez et al. 2014a). The only other identified kinorhynch from abyssal depths is *Campyloderes* cf. *vanhöffeni* Zelinka, 1913 that was recorded at 5,055, 5,102, and 5,118 m southeast of the Canary Islands and at 5,064 m in the Guinea Basin (Neuhaus and Sørensen 2013)—a locality near the study site of the present contribution.

Among the homalorhagid genera, *Pycnophyes* is the most species-rich so far, with 54 described species (Neuhaus 2013; Sánchez et al. 2013, 2014a; Sørensen 2013). *Pycnophyes* species are characterized by having one tergal and three sternal plates on segment 1, one tergal and two sternal plates on segments 2–11, and one pair of lateral terminal spines on segment 11 (Higgins 1983; Sørensen and Pardos 2008; Zelinka 1928). Taxonomically relevant characters include presence and position of setae, subcuticular structures, and middorsal processes or elevations (Kristensen and Higgins 1991; Sánchez et al. 2011; Sørensen and Pardos 2008). Apart from their internal organs, males usually differ from females by the presence of penile spines on segment 11 and two large ventral tubes on segment 2 (Higgins 1983; Zelinka 1928). However, the latter character is not always present (Neuhaus 2013; Sánchez et al. 2014a). Until now, the lack of this pair of ventral tubes has been reported in the description of five species only (Higgins 1966, 1983; Lang 1953; Sánchez et al. 2011).

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In the present study, we describe two new species of *Pycnophyes* from the Guinea Basin (Atlantic Ocean), collected at 5,136 to 5,174 m depth. This contribution increases our extremely limited knowledge about abyssal kinorhynchs and focuses the attention on the peculiar group of *Pycnophyes* species without ventral tubes on segment 2 in males.

Materials and methods

The specimens analyzed in the present study were collected during the R/V Meteor Diva2 M63/2 Cruise, carried out from February to the end of March 2005. Deep-sea samples (over 5,000 m depth) were collected along the Mid- and East Atlantic region using a multicorer. Specimens studied herein were collected in the Guinea Basin on March 23, DIVA2 Station M63/2 98, position 00°37′12″N, 006°28′06″W, at 5,165 to 5,174 m depth, and on March 19, 2005, DIVA2 Station M63/2 79, position 00°50′00″N, 005°35′00″W, at 5,136 to 5,142 m depth (Fig. 1).

The upper 5 cm of sediment from one core in each multicore sample was fixed in a 5 % formaldehyde solution. Later, fixed samples were washed with freshwater in a 40-μm sieve at the laboratory of the Senckenberg Research Institute (Wilhelmshaven, Germany). Meiofauna was extracted using centrifugation with the colloidal silica polymer Levasil (Neuhaus and Blasche 2006), sorted to main groups, and then distributed among relevant experts.

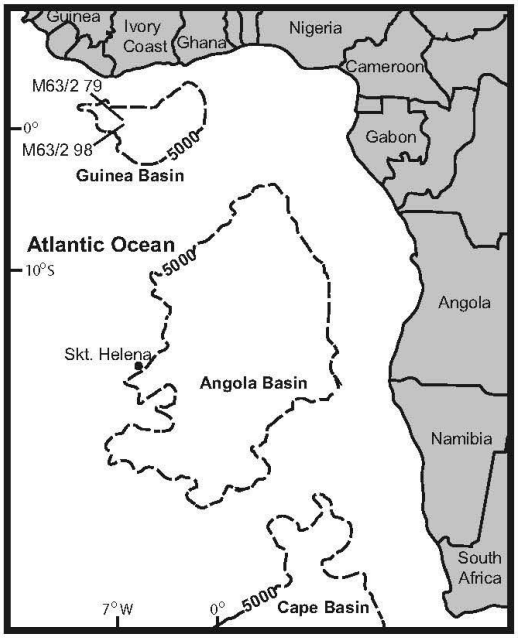

Fig. 1 Map showing the sampling areas of R/V Meteor and the two sampling localities, M63/2 79 and M63/2 98

All specimens of the two new species were prepared for light microscopy (LM), transferred from formaldehyde solution to water, and then dehydrated through a graded series of glycerin. The specimens were kept overnight in 100 % glycerin and then mounted in Fluoromount-G® on a glass slide and sealed with Glyceel®. These specimens were examined with an Olympus BX51 compound microscope equipped with DIC optics, photographed with Olympus DP20 and DP70 cameras, and measured with Cell^D software. Line art figures were made with Adobe Illustrator CS4.

The general terminology for kinorhynchs follows Sørensen and Pardos (2008). Terminology related to seta distribution for homalorhagids follows the standardization criteria established by Sánchez et al. (2011) and adopted by all authors (Herranz et al. 2014; Sánchez et al. 2013, 2014a, b; Sørensen et al. 2010; Yamasaki et al. 2012), except Neuhaus (2013).

Results

Taxonomic account

Order Homalorhagida (Zelinka, 1896) Higgins, 1964

Family Pycnophyidae Zelinka, 1896

Genus *Pycnophyes* Zelinka, 1907

Pycnophyes nubilis sp. nov

(Figs. 2–3)

Type material Holotype, adult female, collected on March 23, 2005 in the Guinea Basin at DIVA Station M63/2 98, position 00°37′12″N, 006°28′06″W, from mud at 5,165 to 5,174 m depth; mounted in Fluoromount-G®; and deposited at the Museum für Naturkunde (Berlin, Germany) under accession number ZMB 11521. No additional specimens were available.

Diagnosis *Pycnophyes* with middorsal processes on segments 1–10 and paradorsal intracuticular atria on segments 1–9; middorsal processes of similar size until segment 7 and, from this point, turning progressively longer towards the posterior trunk segments; middorsal process on segment 10 extending over segment 11; pairs of paradorsal setae on segment 1 only, laterodorsal setae on segments 2–9, paralateral setae on segment 1, lateroventral setae on segments 2, 4, 6, 8, and 10 (two pairs); ventrolateral setae on segments 1 and 5 and ventromedial setae on segments 2–9; and one pair of horn-shaped cuticular extensions on segment 11.

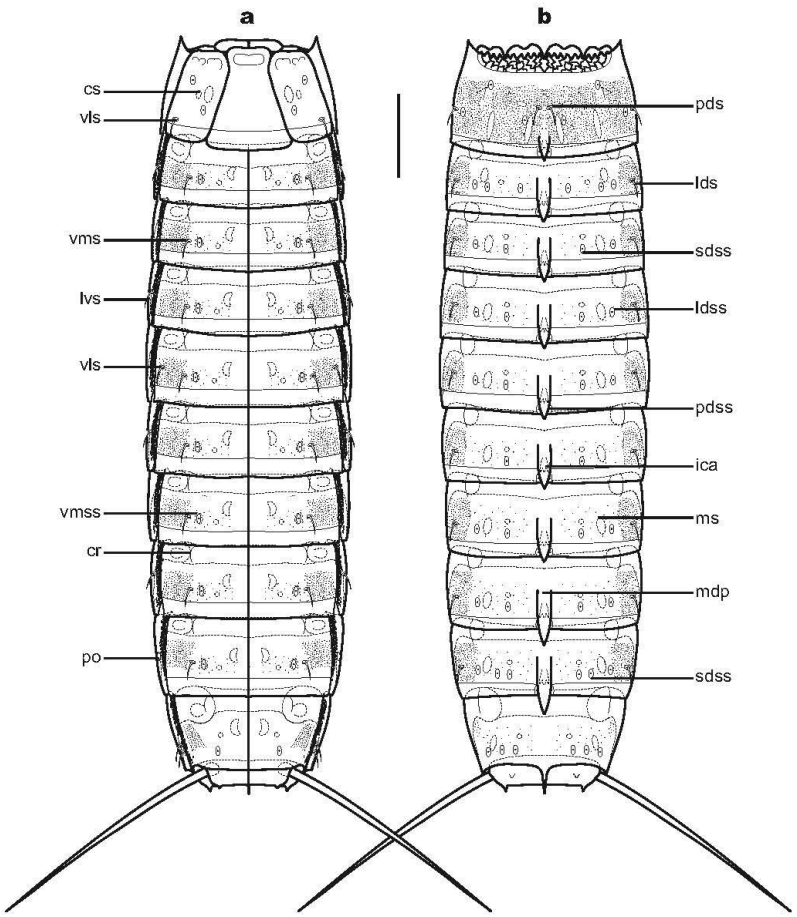

Fig. 2 Line art illustrations of *P. nubilis* sp. nov. **a** Female, ventral view. **b** Female, dorsal view. *Scale bar* 100 μm. *cr* cuticular ridge, *cs* cuticular scar, *ica* intracuticular atria, *lds* laterodorsal seta, *ldss* laterodorsal sensory spot, *lvs* lateroventral seta, *mdp* middorsal process, *ms* muscular scar, *pds* paradorsal seta, *pdss* paradorsal sensory spot, *po* protonephridial opening, *sdss* subdorsal sensory spot, *vls* ventrolateral seta, *vms* ventromedial seta, *vmss* ventromedial sensory spot

Etymology The species name, *nubilis*, is Latin for "marriageable" and refers to the female nature of the holotype and the lack of known male specimens.

Description

All dimensions and measurements are summarized in Table 1 and distribution of sensory spots and setae in Table 2.

Introvert and mouth cone Mouth cone with nine outer oral styles of similar size, long, thin, and flexible. The introvert has six rings of spinoscalids plus an additional ring of trichoscalids, with seven dorsal and seven ventral ones. Ten primary spinoscalids composed of two units are present in ring 01. Detailed data on the distribution of other spinoscalids were not available.

Neck Neck with dorsal and four ventral placids, all of them strongly sclerotized and with concave surfaces. Dorsal placids are rectangular, of similar size, and with an apical, medial notch (Figs. 2b and 3c). The two medial ventral placids are wider and higher than the lateral ones (Fig. 3d).

Trunk Trunk composed of 11 segments. The first segment consists of one tergal and three sternal plates, including two episternal and one trapezoidal midsternal plate (Figs. 2a, b and 3a, b). The remaining segments have one tergal and two sternal plates (Figs. 2a, b and 3a, b). The segment width is nearly constant throughout the trunk. Maximum sternal width is reached at segment 8, tapering slightly from this segment towards the terminal one. Segments 1 to 10 have middorsal processes visibly extending beyond the posterior margin of the respective segment (Fig. 3b). These middorsal processes are of similar size until segment 7 and turn progressively

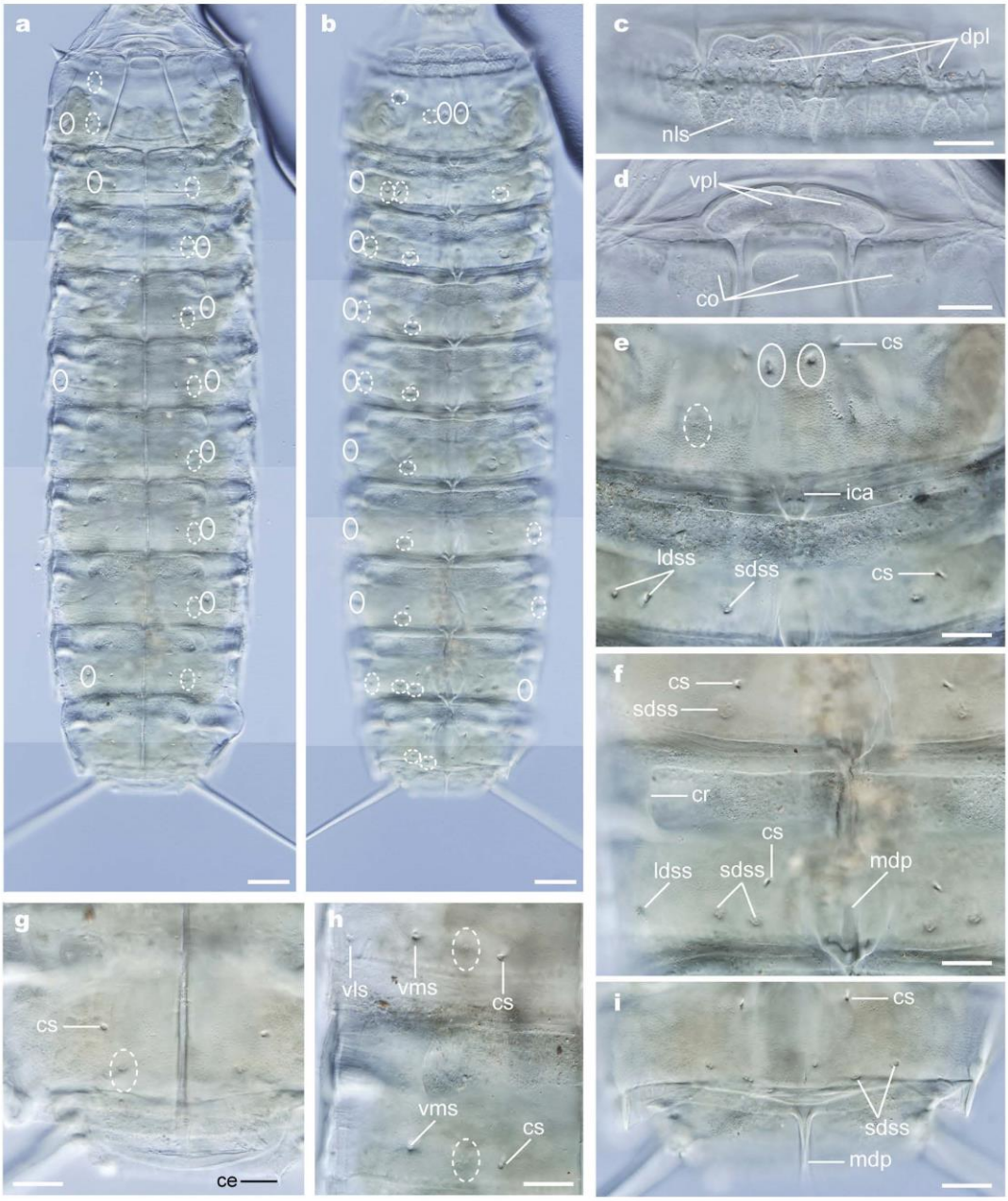

Fig. 3 Holotypic female of *P. nubilis* sp. nov. Differential interference contrast photographs. **a** Ventral view. **b** Dorsal view. **c** Dorsal placids and dorsal anterior margin of segment 1. **d** Ventral placids and ventral anterior margin of segment 1. **e** Dorsal view of segments 1 and 2. **f** Dorsal view of segments 8 and 9. **g** Ventral view of segments 10 and 11. **h** Right sternal plates of segments 5 and 6. **i** Dorsal view of segment 10. *Scale bar* **a** and **b** 50 µm, **c**–**i** 20 µm. *ce* cuticular extension, horn-shaped, *co* cuticular ornamentation, *cr* cuticular ridge, *cs* cuticular scar, *dpl* dorsal placids, *ica* intracuticular atria, *ldss* laterodorsal sensory spot, *mdp* middorsal process, *nls* net-like structure, *sdss* subdorsal sensory spot, *vls* ventrolateral seta, *vms* ventromedial seta, *vpl* ventral placids. *Dotted circles* indicate sensory spots. *Solid circlets* indicate setae

Table 1 Measurements (micrometers) of female holotype of *P. nubilis* sp. nov.

Character	Holotype ♀
tl	897
sw10	163
msw-8	202
msw/tl	22.5 %
lts/tl	29.3 %
s1	125
s2	71
s3	84
s4	86
s5	89
s6	91
s7	95
s8	93
s9	93
s10	100
s11	34
lts	263
mdp8	16
mdp9	16
mdp10	25

lts lateral terminal spine, *mdp8-mdp10* middorsal process lengths of segments 8–10, *msw-8* maximum sternal width of segment 8, *s1–s11* segment lengths of trunk segments 1–11, *sw10* sternal width of trunk segment 10, *tl* trunk length

longer posteriorly, reaching their maximum length on segments 9 and 10. Middorsal processes on segments 1–9 with paired paradorsal intracuticular atria (Fig. 2b). Trunk surface is perforated by numerous cuticular pores. Pairs of oval muscular scars are in laterodorsal and paraventral positions on segments 2–10 and ventromedial ones on segment 1 (Fig. 2a, b). Pairs of cuticular scars (likely outlets of gland cells) are in subdorsal and ventromedial positions on segments 1–10 (Fig. 2a, b), oval and oblique on segment 1, and rounded

Table 2 Summary of location of setae and sensory spots in *P. nubilis* sp. nov. arranged by series

Segment	PD	SD	LD	PL	LV	VL	VM
1	se,ss	ss	ss,ss	se		se	ss,ss
2	ss	ss	ss,ss,se		se		ss,se
3	ss	ss	ss,se				ss,se
4	ss	ss	ss,se		se		ss,se
5	ss	ss	ss,se			ss	ss,se
6	ss	ss	se		se		ss,se
7	ss	ss	ss,se				ss,se
8	ss	ss	ss,se		se		ss,se
9	ss	ss,ss	ss,se				ss,se
10		ss,ss	ss		se,se		ss
11		ss3			lts		ss3

LD laterodorsal, *lts* lateral terminal spines, *LV* lateroventral, *PD* paradorsal, *PL* paralateral, *SD* subdorsal, *se* setae, *ss* sensory spots, *ss3* sensory spots type 3, *VL* ventrolateral, *VM* ventromedial

on the remaining segments. Segments 2–10 have hairy tergosternal junctions and pairs of laterodorsal and ventromedial cuticular ridges (Fig. 2a, b). Conspicuous peg and socket joints and pachycycli, both more developed on segment 10 (Fig. 2a, b). Pectinate fringe and secondary pectinate fringes are not visible with LM. Apodemes are not present (Fig. 3b).

Segment 1 Segment with anterolateral margins of tergal plate projecting into horn-shaped extensions. The anterior edge of tergal plate is strongly denticulated, followed by a narrow area of cuticular ornamentation as a net-like structure (Fig. 3c). Middorsal process flanked by one pair of paradorsal setae and sensory spots, the latter being associated with intracuticular atria (Fig. 3e). One pair of paralateral setae appears to be present. Three additional pairs of sensory spots are present on the tergal plate, one in subdorsal and two in laterodorsal positions (Figs. 2b and 3e). The subdorsal pair and the most lateral one are located in the middle region of the plate; the other laterodorsal pair is anterior to them. The subdorsal cuticular scars are located in the middle region of the plate (Fig. 3e), more mesial than those of the remaining segments and anterior to the subdorsal sensory spots. Two pairs of elongate and oblique cuticular scars, one subdorsal and one laterodorsal, are present (Fig. 2b). Ventral anterior margin is ornamented, followed by a depressed area (Fig. 3d). A pair of ventrolateral setae is present near the posterior margin of the episternal plates. Two pairs of ventromedial sensory spots present, one located on the anterior third and one on the posterior third, more mesially. Pairs of ventromedial cuticular and muscular scars present in the middle region of the plate, between the sensory spots (Fig. 2a).

Segment 2 Tergal plate with middorsal process flanked by one pair of paradorsal sensory spots associated with intracuticular atria similar to those on the preceding segment. One pair of laterodorsal and lateroventral setae present. Three additional pairs of sensory spots present, one in subdorsal and two in laterodorsal positions (Fig. 3e). The laterodorsal pair, located most laterally, is aligned with the laterodorsal cuticular ridges (Fig. 2b). Subdorsal cuticular scars located anterior and between the subdorsal and the most mesial laterodorsal sensory spots (Fig. 3e). Sternal plates with one pair of ventromedial setae, sensory spots, and cuticular scars. The ventromedial setae appear more lateral than the sensory spots and are aligned with the ventromedial cuticular ridges. The ventromedial cuticular scars are located mesially to the ventromedial sensory spots (Fig. 2a).

Segment 3 Tergal plate similar to that of segment 2, but without lateroventral setae and with only one pair of laterodorsal sensory spots. The subdorsal pair of sensory spots is laterally displaced and aligned with the subdorsal cuticular scars (Fig. 2b). Sternal plates as those on segment 2.

Segment 4 Tergal and sternal plates similar to those on segment 3, but with one pair of lateroventral setae (Fig. 2a).

Segment 5 Tergal and sternal plates similar to those on segment 3, but with one pair of ventrolateral setae on sternal plates (Fig. 3h).

Segment 6 Tergal plate similar to that on segment 4, but without laterodorsal sensory spots (Fig. 2b). Sternal plates similar to those on segment 3 (Fig. 3h).

Segment 7 Tergal and sternal plates similar to those on segment 3.

Segment 8 Tergal plate similar to that on segment 4, but with the middorsal process slightly more developed than that on the preceding segment (Fig. 3f). Sternal plates similar to those on segment 3.

Segment 9 Tergal and sternal plates similar to those on segment 3, but with two pairs of subdorsal sensory spots (Fig. 3f). The most mesial pair of sensory spots is aligned with the subdorsal one of the preceding segment, while the other pair is located more laterally (Fig. 2b). Middorsal process slightly more developed than the one on segment 8, surpassing the first third of the following segment (Fig. 2b). Paralateral protonephridial openings are present, not sieve-like.

Segment 10 Middorsal process acicular, appearing much thinner than those on preceding segments, but conspicuously extending over the terminal trunk segment (Fig. 3i). Paradorsal sensory spots and intracuticular atria absent (Fig. 3i). Three pairs of sensory spots located near the posterior margin of the segment, two in subdorsal and one in laterodorsal positions (Fig. 3i). The subdorsal pairs are aligned with the subdorsal ones of the preceding segment. Two pairs of lateroventral setae present. Sternal plates have one pair of ventromedial sensory spots and cuticular scars (Fig. 3g). The posterior ventral margin of the segment straight (Fig. 3g).

Segment 11 Middorsal process absent. One pair of long lateral terminal spines present. Tergal plate with one pair of subdorsal type 3 sensory spots and one pair of conspicuous horn-shaped lateral cuticular extensions (Fig. 3g).

Associated kinorhynch fauna

Besides the two new species of *Pycnophyes* described herein, the holotype of *M. abyssalis* and about ten specimens of *Echinoderes* were recorded from the same station. The *Echinoderes* specimens have not yet been described or examined any further.

Remarks

P. nubilis sp. nov. can be distinguished from most species of the genus by its well-developed middorsal processes. These keel-shaped projections clearly surpass the posterior margins of their segments and increase in length towards the posterior segments. The longest ones are those of segments 9 and 10, exceeding the first third of segment 10 and extending over the terminal trunk segment, respectively. Seven other species in the genus have similar middorsal processes progressively increasing in lengths towards the posterior segments: *Pycnophyes arctous* Adrianov, 1999; *Pycnophyes carinatus* Zelinka, 1928; *Pycnophyes chilensis* Lang, 1953; *Pycnophyes chukchiensis* Higgins, 1991; *Pycnophyes cristatus* Sánchez et al., 2013; *Pycnophyes furugelmi* Adrianov, 1999; and *Pycnophyes odhneri* Lang, 1949 (see Adrianov and Malakhov 1999; Higgins 1991; Lang 1949, 1953; Sánchez et al. 2013; Zelinka 1928). However, only four of these species possess a middorsal process on segment 10 conspicuously extending over the last trunk segment: *P. arctous*, *P. chukchiensis*, *P. cristatus*, and *P. furugelmi* (see Adrianov and Malakhov 1999; Higgins 1991; Sánchez et al. 2013).

P. arctous is easily distinguished from *P. nubilis* sp. nov. by the appearance of its obtuse and blunt middorsal processes on segments 1–7 that differ from the much more conspicuously pointed ones in *P. nubilis* sp. nov. Moreover, *P. arctous*, according to the literature, lacks laterodorsal and ventromedial setae, cuticular ornamentation in the anteriormost tergal region of segment 1, and notches on its placids (Adrianov and Malakhov 1999) (ventrolateral setae on segment 5 and paraventral setae on segments 3 and 7–9 seem to be present, Sánchez, personal observation); all characters are present in *P. nubilis* sp. nov.

P. furugelmi has a large middorsal process on segment 10, cuticular ornamentation in the anteriormost region of the first segment, placids with notches in the middle region, and one pair of horn-shaped cuticular extensions on segment 11. However, its middorsal processes are not as well-developed as those of *P. nubilis* sp. nov., especially not on segment 9. Moreover, *P. furugelmi* has ventromedial sensory spots only (males on segments 3–8 and females on 3–6 and 9), whereas the new species has also sensory spots throughout the whole dorsal side (for a detailed distribution see Table 2). In addition, *P. furugelmi* has a different ventromedial and laterodorsal seta distribution (males on segments 2–8 and on 4–8; females on segments 2–6 and on 2, 3, 4, 6, and 8) (Adrianov and Malakhov 1999) than that of the new species (both sexes with ventromedial and laterodorsal setae on segments 2–9).

P. cristatus resembles the new species in several ways, such as its middorsal processes, cuticular ornamentation in the anteriormost region of the first segment, placids with notches in the middle region, horn-shaped cuticular extensions on segment 11, lateroventral seta distribution, as well as a similar

sensory spot distribution. However, *P. cristatus* differs from the new species by the absence of laterodorsal and ventromedial setae on segments 2–6 as well as paradorsal setae on segment 1 (Sánchez et al. 2013). Additional features to discriminate between both species are as follows: lateral terminal spines/trunk length (lts/tl) proportion is higher in *P. nubilis* sp. nov. than in *P. cristatus* (29 and 24 %, respectively) and middorsal processes on segments 9 and 10 are longer in *P. cristatus* than in *P. nubilis* sp. nov. (32 and 50 μm vs. 16 and 25 μm, respectively); maximum trunk width is reached on segment 6 for *P. cristatus* and on segment 8 for *P. nubilis* sp. nov.

P. chukchiensis and *P. nubilis* sp. nov. have the same distribution of laterodorsal and ventromedial setae, and both show ventrolateral setae on segment 5 and horn-shaped cuticular extensions on segment 11. However, *P. chukchiensis* has ventrolateral setae on segment 10 and lateroventral setae on segments 2–10, whereas *P. nubilis* sp. nov. lacks both ventrolateral setae on segment 10 and lateroventral setae on segments 3, 5, 7, and 9. Moreover, both species differ by their distribution of sensory spots, since *P. chukchiensis* has ventromedial sensory spots only, whereas *P. nubilis* sp. nov. also has laterodorsal ones. Finally, the new species has paradorsal setae on segment 1, which are absent in *P. chukchiensis* (see Higgins 1991).

Pycnophyes farinellii sp. nov

(Figs. 4 and 5)

Type material Holotype, adult female, collected on March 23, 2005 in the Guinea Basin at DIVA Station M63/2 98, position 00°37′12″N, 006°28′06″W, from mud at 5,165 to 5,174 m depth; mounted in Fluoromount-G®; and deposited at the Museum für Naturkunde (Berlin, Germany) under accession number ZMB 11522. Paratype, adult male were collected on March 19, 2005 in the Guinea Basin at DIVA Station M63/2 79 (Fig. 1), position 00°50′00″N, 005°35′00″W, from mud at 5,136 to 5,142 m depth; mounted in Fluoromount-G®; and deposited at the Museum für Naturkunde under accession number ZMB 11523.

Diagnosis Pycnophyes with middorsal elevations on segments 2–9; anterior margin of tergal and sternal plates of segment 1 ornamented; and pairs of laterodorsal setae on segments 2 and 9, paralateral setae on segment 1, lateroventral setae on segments 2, 4, 6, and 8, ventrolateral setae on segments 5 and 10, and ventromedial setae on segments 2 and 9. Males do not have ventromedial tubes on segment 2.

Etymology The species name refers to Farinelli, a celebrated Italian castrato singer of the eighteenth century, with reference to the species' lack of conspicuous ventral tubes normally present on segment 2 of male *Pycnophyes*—a typical sexual character of the genus.

Description

All dimensions and measurements are summarized in Table 3 and distribution of sensory spots and setae in Table 4.

Introvert and mouth cone Mouth cone with nine outer oral styles of similar size, long, thin, and flexible. Introvert with six rings of spinoscalids plus and additional ring of trichoscalids. Spinoscalid number and arrangement are apparently identical to the pattern described for *P. nubilis* sp. nov.

Neck Four dorsal and two ventral placids present (Fig. 5d), all of them are strongly sclerotized, of similar size, and rectangular in shape.

Trunk Trunk with 11 segments. The first one consists of one tergal and three sternal plates, two episternal and one trapezoidal midsternal plate with an Erlenmeyer flask profile (Figs. 4a, b and 5a, b). Segments 2–11 have one tergal and two ventral sternal plates (Figs. 4a, b and 5a, b). The segment width is nearly constant throughout the trunk. Maximum sternal width is reached at segment 6, tapering slightly from this point and towards the posterior end. Segments 2–9 have middorsal elevations, never extending beyond the posterior margin of the segment (Fig. 4b). Paradorsal intracuticular atria is flanking the middorsal elevations on segments 2–8 (Fig. 4b). Segments 2–9 have oval muscular scars in laterodorsal and paraventral positions (Fig. 4a, b) (muscular scars not visible on segment 10). Similar muscular scars present on segment 1, but in laterodorsal and ventromedial positions. Segments 1–10 have two pairs of small cuticular scars, one in subdorsal (horizontal and elongate, Fig. 4b) and one in ventromedial (rounded, Fig. 4a) positions, likely outlets of gland cells. Hairy tergosternal junctions present on segments 2–10. Segments 2–10 with pairs of laterodorsal, ventrolateral, and ventromedial cuticular ridges (Fig. 4a, b). Pachycycli and peg and socket joints are well-developed and of similar size from segment 2 to segment 10. Conspicuous thickenings of the pachycycli present on segment 10 (Fig. 5a), between the cuticular ridges. Pectinate fringe and secondary pectinate fringes are not visible with LM. Apodemes are not present (Fig. 5a).

Segment 1 Segment with anterolateral margins of tergal plate projecting into horn-shaped extensions. Anterior edge of tergal plate weakly denticulated, followed by a narrow reticulated area (Fig. 5c). Longitudinal cuticular thickenings present in laterodorsal position (Fig. 5c). Setae present in paralateral positions. Three pairs of sensory spots present, two in subdorsal and one in laterodorsal positions (Fig. 4b). The

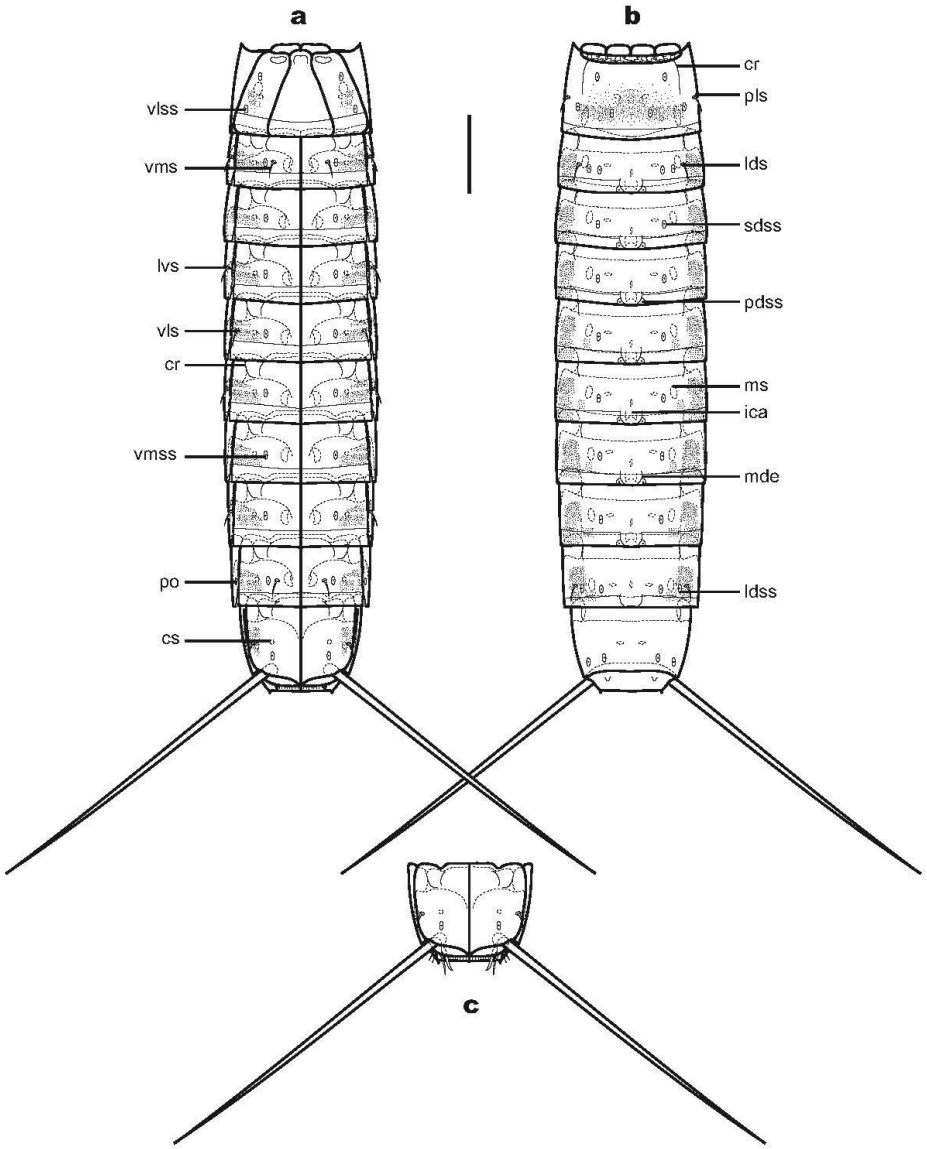

Fig. 4 Line art illustrations of *P. farinellii* sp. nov. **a** Female, ventral view. **b** Female, dorsal view. **c** Male, segments 10 and 11, ventral view. *Scale bar* 100 μm. *cr* cuticular ridge, *cs* cuticular scar, *ica* intracuticular atria, *lds* laterodorsal seta, *ldss* laterodorsal sensory spot, *lvs* lateroventral seta, *mde* middorsal elevation, *ms* muscular scar, *pdss* paradorsal sensory spot, *pls* paralateral seta, *po* protonephridial opening, *sdss* subdorsal sensory spot, *vls* ventrolateral seta, *vlss* ventrolateral sensory spot, *vms* ventromedial seta, *vmss* ventromedial sensory spot

ventral anterior margin has a depressed area on both episternal and midsternal plates (Fig. 5d). Pairs of sensory spots are present in ventrolateral and ventromedial positions. The ventrolateral sensory spots are located on the posterior third of the segment and the ventromedial one on the anterior third (Fig. 4a).

Segment 2 Middorsal elevation present, flanked by a pair of paradorsal sensory spots associated with intracuticular atria (Fig. 4b). Single middorsal intracuticular structure located anterior to the middorsal elevation. Pairs of laterodorsal and lateroventral setae present. The laterodorsal pair appears located slightly more mesially than the laterodorsal pair of

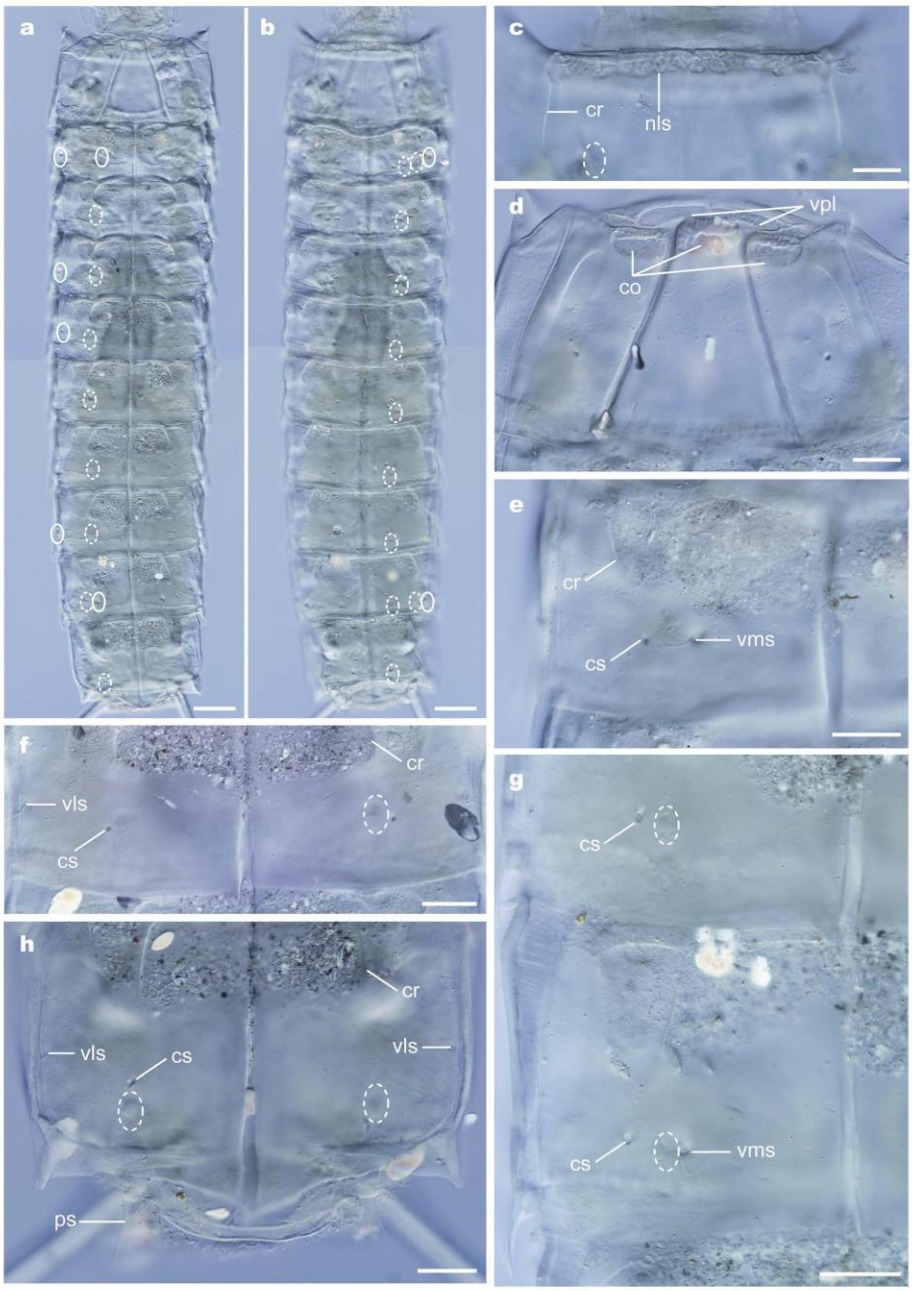

Fig. 5 *P. farinellii* sp. nov. Differential interference contrast photographs. **a–c** and **g** Holotypic female. **d–f** and **h** Paratypic male. **a** Ventral view. **b** Dorsal view. **c** Dorsal view of anterior margin of segment 1. **d** Ventral view of segment 1. **e** Right sternal plates of segment 2. **f** Ventral view of segment 5. **g** Right sternal plates of segments 8 and 9. **h** Ventral view of segments 10 and 11. *Scale bar* **a** and **b** 50 μm; **c–h** 20 μm. *co* cuticular ornamentation, *cr* cuticular ridge, *cs* cuticular scar, *nls* net-like structure, *ps* penile spine, *vls* ventrolateral seta, *vms* ventromedial seta, *vpl* ventral placids. *Dotted circles* indicate sensory spots. *Solid circlets* indicate setae

Table 3 Measurements (micrometers) of female holotype and male paratype of *P. farinellii* sp. nov.

Character	Holotype ♀	Paratype ♂
tl	792	805
sw10	135	140
msw-6	158	158
msw/tl	19.6 %	20.1 %
lts/tl	41.3 %	50 %
s1	108	96
s2	75	70
s3	77	85
s4	77	80
s5	85	81
s6	85	86
s7	84	90
s8	84	92
s9	82	88
s10	109	111
s11	38	31
lts	327	401

lts lateral terminal spine, *msw-6* maximum sternal width of segment 6, *s1–s11* segment lengths of trunk segments 1–11, *sw10* sternal width of trunk segment 10, *tl* trunk length

cuticular ridges (Fig. 4b). Two additional pairs of sensory spots, one in subdorsal and one in laterodorsal positions, present (Fig. 4b). Sternal plates with pairs of ventromedial sensory spots and cuticular scars. The ventromedial pair of sensory spots is located more mesially than the cuticular scars (Fig. 4a). Both sexes with a pair of ventromedial setae, located in a more mesial position in respect to the sensory spots (Fig. 5e). Males without ventromedial tubes (Fig. 5e).

Segment 3 Middorsal elevation present, flanked by a pair of paradorsal sensory spots associated with intracuticular atria. Single middorsal intracuticular structure located anterior to the middorsal elevation. Additional sensory spots present in subdorsal positions only, aligned with the subdorsal ones of the preceding segment (Fig. 4b). Setae are not present. Sternal plates similar to those of segment 2, but without ventromedial setae (Fig. 4a).

Segment 4 Tergal and sternal plates similar to those on segment 3, but with one pair of lateroventral setae.

Segment 5 Tergal and sternal plates similar to those on segment 3, but with one pair of ventrolateral setae (Fig. 5f).

Segment 6 Segment similar to segment 4.

Segment 7 Tergal and sternal plates similar to those on segment 3.

Segment 8 Segment similar to segment 4 (Fig. 5g).

Segment 9 Tergal plate similar to that on segment 2, but without paradorsal sensory spots and with the laterodorsal setae and sensory spots laterally displaced (Fig. 4b). Paralateral protonephridial openings are present, not sieve-like. Sternal plates similar to those on segment 2 (Fig. 5g).

Segment 10 Middorsal process and associated structures absent. Two pairs of sensory spots located near the posterior margin of the segment, one in subdorsal and one in laterodorsal positions. Sternal plates with one pair of ventrolateral setae. Pairs of ventromedial sensory spots and cuticular scars present (Fig. 5h), both aligned with the ventromedial sensory spots of the preceding segment. The posterior ventral margin of the segment is pointed (Fig. 5h).

Table 4 Summary of location of setae and sensory spots in *P. farinellii* sp. nov. arranged by series. *LD* laterodorsal, *lts* lateral terminal spines, *LV* lateroventral, *m* male condition of sexually dimorphic character, *PD* paradorsal, *PL* paralateral, *ps* penile spines, *SD* subdorsal, *se* setae, *ss* sensory spots, *ss3* sensory spots type 3, *VL* ventrolateral, *VM* ventromedial

Segment	PD	SD	LD	PL	LV	VL	VM
1		ss,ss	ss	se		ss	ss
2	ss	ss	ss,se		se		ss,se
3	ss	ss					ss
4	ss	ss			se		ss
5	ss	ss				se	ss
6	ss	ss			se		ss
7	ss	ss					ss
8	ss	ss			se		ss
9		ss	ss,se				ss,se
10		ss	ss			se	ss
11		ss3		ps,ps (m)	lts		

Segment 11 Segment with one pair of long lateral terminal spines. Tergal plate with one pair of subdorsal type 3 sensory spots. Ventral segment margin hairy. Males with two pairs of penile spines (Fig. 5h).

Associated kinorhynch fauna

At its type locality, the new species co-occurs with *P. nubilis* sp. nov., *M. abyssalis*, and some unidentified species of *Echinoderes*. On the paratype locality, Station M63/2 79, additional kinorhynchs included *M. abyssalis*, a single species of *Echinoderes*, and specimens of a cyclorhagid genus resembling *Antygomonas* or *Sphenoderes*.

Remarks

P. farinellii sp. nov. can be easily discriminated from most other species of the genus by its scarcity of setae. The few setae (excluding the lateroventral ones) are distributed as follows: paralaterally on segment 1, laterodorsally and ventromedially on segments 2 and 9, and ventrolaterally on segments 5 and 10. Many species of *Pycnophyes* share the absence of middorsal, paradorsal, and subdorsal setae on all trunk segments with *P. farinellii* sp. nov., but only five of them also show a scarcity of setae in laterodorsal positions and throughout the whole ventral side. These include *Pycnophyes argentinensis* Martorelli and Higgins, 2004; *P. arctous*; *P. chilensis*; *P. cristatus*; and *Pycnophyes smaug* Sánchez et al., 2013 (see Adrianov and Malakhov 1999; Lang 1953; Martorelli and Higgins 2004; Sánchez et al. 2013, personal observation; Zelinka 1928).

However, *P. arctous*, *P. chilensis,* and *P. cristatus* have conspicuous middorsal processes on most trunk segments. These structures are robust, keel-shaped, and clearly extending beyond the posterior margin of the segment (Lang 1953; Adrianov and Malakhov 1999; Sánchez et al. 2013). *P. farinellii* sp. nov. does not present similar processes, but has middorsal elevations along the trunk.

P. argentinensis has ventral setae on segments 2 and 9, as *P. farinellii* sp. nov., but both species present a different dorsal seta distribution. *P. argentinensis* has lateroventral setae on segments 2, 4, and 6 and laterodorsal setae, at least, on segments 3 and 4 (Martorelli and Higgins 2004; Sánchez, personal observation). The new species has lateroventral setae on segment 8 also and laterodorsal setae on segments 2 and 9 only.

P. smaug resembles the new species in many ways, such as presence of middorsal elevations on segments 2–9; laterodorsal setae on segments 2 and 9 only; lateroventral setae on segments 2, 4, 6, and 8; ventrolateral setae on segment 5; and ventromedial setae on segment 9 (Sánchez et al. 2013). However, *P. smaug* has a middorsal elevation on segment 1 and lateroventral setae on segment 10. Both characters are absent in *P. farinellii* sp. nov.

The absence of conspicuous ventral tubes on segment 2 in males is a noteworthy character condition, since these tubes are a common male character in most species of Pycnophyidae, i.e. all *Kinorhynchus* and most species of *Pycnophyes* (Kristensen and Higgins 1991; Neuhaus 2013; Sørensen and Pardos 2008). The absence of these tubes has been reported only from seven other *Pycnophyes* species out of 56 species described so far (comprising the two new species reported in the present study): *P. chilensis*; *Pycnophyes dolichurus* Sánchez et al., 2011; *Pycnophyes ecphantor* Higgins, 1983; *Pycnophyes egyptensis* Higgins, 1966; *Pycnophyes longicornis* Higgins, 1983; *Pycnophyes rugosus* Zelinka, 1928 (Sánchez, personal observation; original description does not give any data about ventral tubes in males); and *Pycnophyes* cf. *ponticus* (Reinhard 1881) (see Lang 1953; Higgins 1966, 1983; Sánchez et al. 2011, 2012). Regarding the latter taxon—which corresponds to *Pycnophyes* sp3 in Sánchez et al. 2012—Reinhard 1885 describes the presence of ventral tubes in males of this species but actually they are absent in all males, 23 specimens, collected from the Gulf of Naples and Spain (Sánchez, personal observation) (Zelinka collected *P. ponticus* in the Gulf of Naples and our observations mostly agree with his description; for detailed information about the confusion on this species, see Higgins 1983; Neuhaus 2013).

The presence of male-specific tubes on segment 2 in species of both *Kinorhynchus* and *Pycnophyes*, and their lack in other species of Homalorhagida as well as in all Cyclorhagida, suggests that the character is autapomorphic for Pycnophyidae. Starting from this consideration, two different hypotheses could be made: (1) The tubes were lost convergently in *P. farinellii* sp. nov. and in the other seven species. (2) The loss happened at a single occasion, hence supporting a close relationship between the eight species. Another possibility would be that the absence of tubes in males is just maintained in these eight species as plesiomorphic homalorhagid trait. However, in this case, *Pycnophyes* would become paraphyletic. Further phylogenetic analyses based on a more extensive Pycnophyidae taxa sampling would test the hypothesis of a closer relationship between the eight species lacking ventral tubes on segment 2.

In addition, two conspicuous and rather uncommon features frequently occur in this special group of *Pycnophyes* species without ventral tubes: the presence of ventral setae on segment 2 in males and relatively long lateral terminal spines in both sexes, measured as lts/tl proportion. Most males of this group of species have ventral setae on segment 2—an uncommon character among the males of *Pycnophyes* (its presence is common in females though). *P. rugosus* is the single species in this group without these ventral setae in males, whereas no data are available to confirm its presence in males of *P. chilensis* (see Lang 1953; Higgins 1966, 1983; Sánchez et al. 2011, personal observation). As for the lateral terminal spines, having

in mind that the average ratio of lts/tl lengths among all species of *Pycnophyes* is 23 % (see Adrianov and Malakhov 1999; Martorelli and Higgins 2004; Sánchez et al. 2011, 2013, 2014b; Yamasaki et al. 2012; Herranz et al. 2014), values equal to or higher than 30 % may be considered as relatively long lateral terminal spines. Out of 56 species of *Pycnophyes*, eight species have this high lts/tl proportion in both sexes. Of these, seven belong to the above-mentioned group lacking ventral tubes in males, while only one species has these tubes, namely *Pycnophyes newguiniensis* Adrianov, 1999 (*P. robustus* Zelinka, 1928 is not included into this latter group since its lts/tl mean values are below 30 %; Sánchez, personal observation). However, *P. newguiniensis* has conspicuously robust lateral terminal spines, being wide along their whole lengths (see Adrianov and Malakhov 1999), whereas in the other seven species, the lateral terminal spines are considerably thinner. The single species with low lts/tl proportions that also lack ventral tubes in males is *P. egyptensis* (see Higgins 1966; Adrianov and Malakhov 1999; Sánchez, personal observation).

Below is a dichotomous short key to facilitate easier identification of species belonging to the group of *Pycnophyes* without ventral tubes on segment 2 in males.

1.	Pairs of twin paraventral setae present on segments 3–7	*P. dolichurus*
	Pairs of twin paraventral setae absent	2
2.	Middorsal processes on segments 1–9	*P. chilensis*
	Middorsal processes absent or, if present, not on all segments 1–9	3
3.	Ventromedial setae present on segments 4–9	4
	Ventromedial setae absent on some segments from segments 4–9	6
4.	All ventromedial setae from segment 4 to segment 9 aligned	*P. ecphantor*
	Some ventromedial setae from segment 4 to segment 9 displaced, not aligned with the remaining ventromedial setae	5
5.	Ventral setae absent on segment 1, all ventromedial setae on segments 3–8 aligned, ventromedial setae of segment 9 mesially displaced	*P. egyptensis*
	Ventral setae present on segment 1, all ventromedial setae on segments 3–9 aligned, except for the setae of segment 8, laterally displaced	*P. longicornis*
6.	Ventral setae absent on segment 1, ventral side with scarcity of setae, ventromedial setae present on segments 2 and 9 only, ventrolateral setae present on segments 5 and 10 only	*P. farinellii* sp. nov.
	Ventrolateral setae present on segment 1, ventral side with ventromedial or ventrolateral setae on segments 2–10	7
7.	Ventrolateral setae present on segment 7, ventromedial on segment 8, peg and socket joints not highly developed, with a normal size	*P. ponticus*
	Ventrolateral setae present on segments 7 and 8, ventromedial setae absent on segment 8, conspicuous peg and socket joints on segments 2–5, highly developed	*P. rugosus*

Conclusions

The description of two new species of *Pycnophyes* from the deep sea increases our general knowledge about homalorhagids and especially our extremely limited information about deep-sea kinorhynchs. Together with the recently described species *M. abyssalis*, these two new species, as well as the reported but yet undescribed associated kinorhynch fauna, show that the deep sea holds an interesting kinorhynch diversity that is still about to be revealed.

The study also points out an interesting and potentially monophyletic group of *Pycnophyes* species with males lacking ventral tubes on segment 2, and suggests that future phylogenetic analyses should also target this character and focus on this hypothesis.

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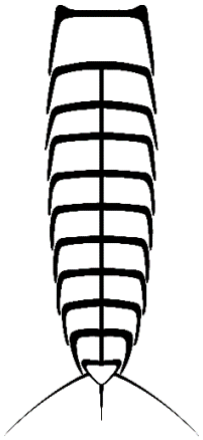
RESULTS

Chapter II

Taxonomy of Neocentrophyidae



A new kinorhynch genus, *Mixtophyes* (Kinorhyncha: Homalorhagida), from the Guinea Basin deep-sea, with new data on the family Neocentrophyidae

Nuria Sánchez, Fernando Pardos, Martin V. Sørensen

RESUMEN: **Un Nuevo género de kinorrincos, *Mixtophyes* (Kinorhyncha: Homalorhagida), de aguas profundidad de la cuenca de Guinea, con nuevos datos sobre la familia Neocentrophyidae.** —Se describe un nuevo género y especie de homalorrágidos, *Mixtophyes abyssalis* gen. et sp. nov., procedente de aguas profundas de la cuenca de Guinea en el océano Atlántico. El nuevo género y especie fue recolectado durante la campaña del R/V Meteor Diva2 M63/2, y se caracteriza por un segmento 1 del tronco formado por una placa tergal y una única placa esternal, sin ningún tipo de división o diferenciación, segmento 2-10 formados por una placa tergal y dos placas esternales y un segmento 11 formado por una placa tergal y una placa esternal. Las espinas lateroterminales están presentes en ambos sexos. Como parte del estudio del nuevo taxón, se reexaminó el material tipo de *Neocentrophyes intermedius* y *N. satyai*, aportándose nueva información para ambas especies. Basándonos en la información obtenida de *Mixtophyes abyssalis* gen. et sp. nov.así como de la re-observación de las dos especies de *Neocentrophyes* se propone una diagnosis enmendada tanto para *Neocentrophyes* como para Neocentrophyidae, y se incluye una clave de identificación actualizada para los géneros y especies de homalorrágidos Neocentrophyidae. *Mixtophyes abyssalis* gen. et sp. nov. es tentativamente asignado a Neocentrophyidae, pero la evaluación de nuestra hipótesis actual sobre la evolución y filogenia de los homalorrágidos indica que la familia Neocentrophyidae es probablemete parafilética y que una revisión de la clasificación de los homalorrágidos es necesaria cuando se disponga de nuevos datos de análisis filogenéticos.

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ORIGINAL ARTICLE

A new kinorhynch genus, *Mixtophyes* (Kinorhyncha: Homalorhagida), from the Guinea Basin deep-sea, with new data on the family Neocentrophyidae

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Abstract A new homalorhagid genus and species, *Mixtophyes abyssalis* gen. et sp. nov., is described from the deep-sea of the Guinea Basin in the Atlantic Ocean. The new genus and species was collected during the R/V Meteor Diva2 M63/2 Cruise, and it is characterized by a trunk with segment 1 consisting of a tergal and a single sternal plate, without any subdivisions or differentiations, segment 2–10 of one tergal and two sternal plates and segment 11 of a tergal and a sternal plate. Lateral terminal spines are present in both sexes. As part of the study of the new taxon, the type material of *Neocentrophyes intermedius* and *N. satyai* was re-examined, and new information is provided for both species. Based on the information from *Mixtophyes abyssalis* gen. et sp. nov. and re-examination of the two *Neocentrophyes* species, emended diagnoses for *Neocentrophyes* and Neocentrophyidae are proposed, and an updated identification key to homalorhagid genera and species of Neocentrophyidae is provided. *Mixtophyes abyssalis* gen. et sp. nov. is tentatively assigned to Neocentrophyidae, but evaluation of our current hypotheses about homalorhagid evolution and phylogeny also indicated that the family most probably is paraphyletic and that revision of homalorhagid classification is required when new results from phylogenetic analyses become available.

Keywords Diva2 · Meiofauna · *Mixtophyes abyssalis* gen. et sp. nov. · *Neocentrophyes* · Taxonomy

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Introduction

Kinorhyncha is a relatively small phylum of meiobenthic invertebrates, with only around two hundred described species (Sørensen 2013). The known species are accommodated in 22 genera which are assigned to two orders, Homalorhagida and Cyclorhagida. According to traditional classification, e.g., Higgins (1990) and Adrianov and Malakhov (1994), the genera are, however, far from been evenly distributed on the two orders. In fact, only four genera, *Kinorhynchus*, *Neocentrophyes*, *Paracentrophyes* and *Pycnophyes*, are assigned to Homalorhagida, whereas the remaining generic diversity belongs to Cyclorhagida. Recent phylogenetic analyses, based on molecular sequence data, indicate though that at least two or three additional genera are more closely related to the homalorhagids (Dal Zotto et al. 2013; Yamasaki et al. 2013), but still a vast majority of the generic and specific diversity is found among the cyclorhagids. Furthermore, among the seven most recently described genera, six of them are clearly cyclorhagids (Neuhaus and Blasche 2006; Sørensen et al. 2007; Sørensen 2008; Sørensen and Rho 2009; Sørensen and Thormar 2010; Herranz et al. 2012), whereas only one, *Franciscideres*, might be a homalorhagid even though its morphology differs considerably from the four currently accepted homalorhagid genera (Dal Zotto et al. 2013).

Hence, discoveries of new homalorhagid genera are rare, and the last description of a certain homalorhagid genus dates more than 30 years from present (Higgins 1983). In the present study, we describe a new kinorhynch species whose general morphology allows an affiliation to Homalorhagida, even though differing from the four known genera, and hence requiring the erection of a new genus. The new species was found at 5,174 m depth in the Guinea Basin (Atlantic Ocean), which also makes it the deepest recorded kinorhynch species that has been named and described. For comparisons, specimens of *Neocentrophyes intermedius* Higgins, 1969 and *N. satyai* Higgins, 1969 were re-examined, and new data for these two species are provided.

Materials and methods

Three specimens of the new kinorhynch genus and species were found in samples collected during the R/V Meteor DIVA2 M63/2 Cruise that lasted from February to the end of March 2005 and collected Mid-Atlantic deep-sea samples from the Northeastern Cape Basin west of South Africa and Angola, in the Northwestern Angola Basin off Angola's coasts, and from several areas in the Guinea Basin, south of the Ivory Coast and Ghana (Fig. 1) (see also Türkay 2005). In all three Basins, the depths exceed 5,000 m.

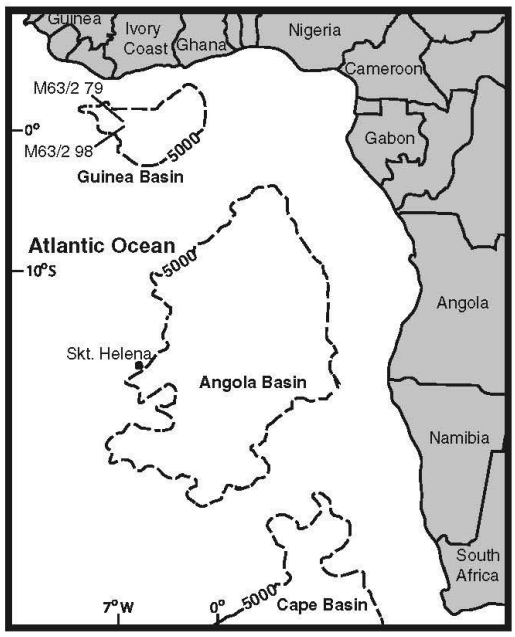


Fig. 1 Map showing the sampling areas of R/V Meteor and the two sampling localities, M63/2 79 and M63/2 98, for *Mixtophyes abyssalis* gen. et sp. nov

The two samples that yielded specimens for the present study were taken with a multicorer in the Guinea Basin at DIVA2 Stations M63/2 79 and M63/2 98, at 5,136–5,142 and 5,165–5,174 m depth, respectively (Fig. 1). The upper 5 cm of sediment from one core in each multicore sample was fixed in 5 % formaldehyde immediately after collecting. Subsequently, in the laboratory at the Senckenberg Research Institute in Wilhelmshaven, Germany, the fixed samples were washed with freshwater in a 40 µm sieve, and meiofauna was extracted from the sediment using centrifugation with the colloidal silica polymer Levasil (see, e.g., Neuhaus and Blasche 2006 for further details). The extracted meiofauna was then sorted to main groups and distributed among some relevant experts.

Of the three specimens representing the new genus and species, two were prepared for light microscopy (LM) and one for scanning electron microscopy (SEM). The specimens for LM were transferred from formaldehyde to water and then dehydrated through a graded series of glycerin. After storage overnight in 100 % glycerin, the specimens were mounted in Fluoromount-G® on a glass slide and sealed with Glyceel®. These specimens, as well as the type material of *Neocentrophyes intermedius* and *N. satyai*, were examined with an Olympus BX51 compound microscope, photographed with Olympus DP20 and DP70 cameras and measured with Cell^D software. Line art figures were made with Adobe Illustrator CS4.

The specimen for SEM was also transferred to water, dehydrated through a graded series of ethanol and then through an ethanol–acetone series, until it was contained in 100 % acetone. Then, the specimen was critical point dried, mounted on a SEM stub, sputter coated with a palladium–platinum mix and examined with a JEOL JSM-6335F field emission scanning electron microscope.

Terminology related to cuticular feature distribution for homalorhagids follows the standardization criteria established by Sánchez et al. (2011) and adopted by most authors (Sánchez et al. 2013, 2014; Sørensen et al. 2010; Yamasaki et al. 2012), except Neuhaus (2013).

The type material of the new species and genus is deposited at the Museum für Naturkunde (MfN), Humboldt-Universität zu Berlin, in Germany. Specimens of *Neocentrophyes intermedius* and *N. satyai* were loaned from the collections of the United States National Museum of Natural History (USNM).

Results

Taxonomic account

Order Homalorhagida (Zelinka, 1896)

Family Neocentrophyidae Higgins, 1969

Genus *Neocentrophyes* Higgins, 1969

Re-examination of *Neocentrophyes intermedius* Higgins, 1969 and *Neocentrophyes satyai* Higgins, 1969

Material examined

All examined specimens of *Neocentrophyes intermedius* were males mounted for LM, females are not known from the species. The specimens are stored at the USNM:

Holotype, male, collected by R. P. Higgins in the Ambatozavavy Bay, Nosy Bé, Madagascar: 13°21.5′S, 48°19.5′E, 1–2 m depth, gray–brown sandy mud, April 15, 1964, accession number RH44.81/USNM37993.

Eight paratypes: four adult males and one juvenile (RH44 series) collected on same date and locality as holotype, accession numbers RH44.76/USNM1207909, RH44.77/USNM1207910, RH44.78/USNM37994, RH44.82/USNM1207912 and RH44.80/USNM1207911 (juvenile); one adult male (RH45 series) collected near type locality at 1–2 m depth, from muddy sand, on April 16, 1964, accession number RH45.14/USNM1207913; two adult males (RH51 series), also near type locality, 18 m depth, gray mud, April 23, 1964, accession numbers RH51.9/USNM1207914 and RH51.10/USNM1207915.

Examined specimens of *Neocentrophyes satyai* were females, mounted for LM, males are not known from the species. The specimens are stored at the USNM: Damaged holotype, female, collected by R. P. Higgins in the Bay of Bengal, India, 10 km off E of Visakhapatnam: 40 m depth, brown sandy mud, March 26, 1964, accession number RH37.87/USNM37995. Two paratypes (RH44 series), two adult females collected on same date and locality as holotype, accession numbers RH37.88/USNM1207916 and RH37.27/USNM1207917, the latter considered as juvenile by Higgins.

Brief descriptions

The examined specimens of both species generally agree with the descriptions made by Higgins (1969); hence, the following report only focuses on additional, not previously

Table 1 Measurements (μm) of type material of *Neocentrophyes*: *N. intermedius* and *N. satyai*. *msw* maximum sternal width, *s1–s11* 1–11 trunk segments length, *sw1–sw11* sternal width of trunk segments 1–11, *TL* trunk length

Character	*Neocentrophyes intermedius*			*Neocentrophyes satyai*		
	Holotype	7 Paratypes		Holotype	2 Paratypes	
		Range	Mean		Range	Mean
TL	624	471–624	549	512	436–441	438
msw	167	138–167	154	152	127–133	130
msw/TL (%)	26.8	24.9–31.6	28.2	29.7	28.9–30.6	29.7
s1	115	92–115	100	–	75	75
s2	41	36–45	39	–	32–33	32
s3	46	42–53	45	–	36–37	36
s4	52	45–53	49	–	36–39	38
s5	55	47–57	52	–	38–39	38
s6	62	51–62	55	–	37–39	38
s7	68	49–69	57	–	41–44	43
s8	68	51–68	57	–	41–47	44
s9	69	50–69	56	–	44–49	46
s10	61	41–61	50	–	41–48	44
s11	26	18–29	24	–	20–23	22
sw1	157	132–157	144	123	115–117	116
sw2	158	132–158	147	137	125–128	126
sw3	161	134–161	148	141	124–132	128
sw4	162	134–162	151	148	126–133	129
sw5	165	133–165	152	150	127–133	130
sw6	166	137–166	153	152	127–133	130
sw7	167	138–167	154	148	126–130	128
sw8	163	139–163	151	147	125–126	126
sw9	156	131–156	144	–	122	122
sw10	126	108–126	116	–	105–106	106
sw11	85	68–86	76	–	68	68

reported information, or cases where our observations are not in concordance with those made by Higgins (1969).

All dimensions and measurements of the examined adult specimens of *Neocentrophyes intermedius* and *N. satyai* are summarized in Table 1 and distribution of sensory spots and setae in Tables 2 and 3, respectively (data on arrangement of sensory spots of *N. satyai* refers to the dorsal side only).

Neck Four dorsal and three ventral placids, with the two ventrolateral ones being really soft and inconspicuous (Fig. 2a). Ventral placids appear elongate (Fig. 2a), whereas dorsal ones are oval (Fig. 3b). Dorsolateral placids are slightly wider than the two central ones.

Table 2 Summary of location of setae, sensory spots, penile spines, midterminal process and cuticular bulbous protrusions in *Neocentrophyes intermedius* arranged by series

Segment	PD	SD	LD	PL	LV	VL	VM	MV
1	se	ss, ss	ss	se		se	ss, ss, ss	
2	se	ss	ss, ss, ss	se			ss, ss	
3	se	ss	ss, ss	se		se	ss, ss	
4	se	ss	ss	se			ss, ss	
5	se	ss	ss, ss	se		se	ss, ss	
6	se	ss	ss	se		se	ss, ss	
7	se	ss	ss	se		se	ss, ss	
8	se	ss	ss	se		se	ss, ss	
9	se	ss	ss, ss	se		se	ss, ss	
10	se	ss	ss	se, ps		se	ss, ss	
11		ss3		ps	cbp		ss3	mtp

cbp cuticular bulbous protrusions of segment 11, *LD* laterodorsal, *LV* lateroventral, *mtp* midterminal process, *MV* midventral, *PD* paradorsal, *PL* paralateral, *ps* penile spines, *SD* subdorsal, *se* setae, *ss* sensory spots (probably type 1), *ss3* sensory spots type 3, *VL* ventrolateral, *VM* ventromedial

Table 3 Summary of location of setae, sensory spots (ventral side only), midterminal process and cuticular bulbous protrusions in *Neocentrophyes satyai* arranged by series

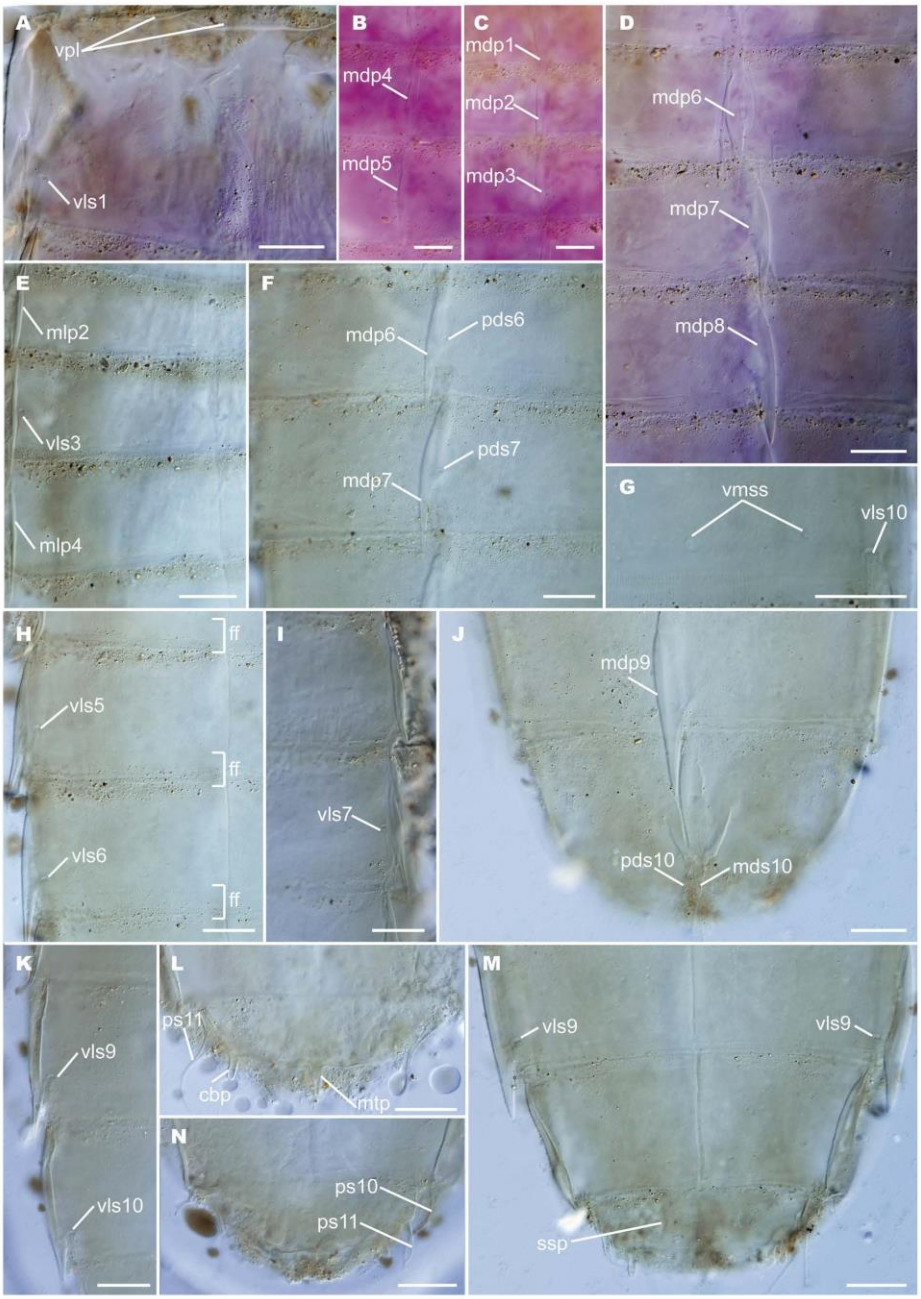
Segment	PD	SD	PL	LV	VL	VM	MV
1	se		se		se	ss, ss	
2	–		se			ss, ss	
3	–		se		se	ss, ss	
4	se		se			ss, ss	
5	se		se		se	ss, ss	
6	se		se		se	ss, ss	
7	se		se		se	ss, ss	
8	se		se		se	ss, ss	
9	–		se		se	ss, ss	
10	–		se		se	ss, ss	
11	–	ss3		cbp		ss3	mtp

cbp cuticular bulbous protrusions of segment 11, *LV* lateroventral, *mtp* midterminal process, *MV* midventral, *PD* paradorsal, *PL* paralateral, *SD* subdorsal, *se* setae, *ss* sensory spots (probably type 1), *ss3* sensory spots type 3, *VL* ventrolateral, *VM* ventromedial—data not available

Fig. 2 *Neocentrophyes intermedius*. Differential interference contrast photographs of males. **a**, **d–m** Paratypic males. **b**, **c** Holotypic male. **a** Ventral view of right half of segment 1. **b** Detail showing middorsal processes on segments 4–5. **c** Detail showing middorsal processes on segments 1–3. **d** Dorsal view of segments 6–8. **e** Ventral view of right half of segments 2–4. **f** Dorsal view of segments 6–7. **g** Left sternal plates on segment 10. **h** Right sternal plates on segments 5–6. **i** Left sternal plates on segments 6–7. **j** Dorsal view on segments 9–11. **k** Right sternal plates on segments 9–10. **l** Dorsal view on segments 10–11. **m** Ventral view on segments 9–11. **n** Segments 10–11, focused medially in specimen. *Scale bar* 20 µm. *cbp* cuticular bulbous protrusion, *ff* free flap, *mdp* middorsal process, *mds* middorsal spine, *mlp* midlateral process, *mtp* midterminal process, *pds* paradorsal seta, *ps* penile spine, *ssp* single sternal plate, *vls* ventrolateral seta, *vmss* ventromedial sensory spot, *vpl* ventral placid. Digits following labels refer to segment numbers

Trunk Segment 1 with one tergal and one sternal plate, not anteriorly divided on the ventral side (Figs. 2a, 3a). Segments 2–10 with one tergal and two sternal plates, and segment 11 with one tergal and a single, undivided sternal plate (Figs. 2m, 3l–m). The segment width is nearly constant throughout the anterior 2/3 of the trunk, but it tapers considerably from segment 8. Maximum width is reached at segment 7 in *Neocentrophyes intermedius* and at segment 6 in *N. satyai*. The cuticle is of medium thickness with pachycycli and peg and socket joints not well developed. Tergosternal junctions on segments 1–9 and 11 present, but not identified on segment 10. These junctions are more easily recognizable at the base of the midlateral spinose processes than in the anteriormost region of the segment (Fig. 3h) (except for segment 1). Inconspicuous ventrolateral cuticular ornamentation presents as longitudinal parallel wrinkles in anteriormost region of the first trunk segment. Elongate and narrow muscular scars on segments 2–10 present in subdorsal (adjacent to the subdorsal sensory spots) and ventromedial positions (adjacent to the mesial sensory spots). These muscular scars stretch over most of the segment length, on both dorsal and ventral sides. Scale-like cuticular hairs are evenly distributed on the segments, except around the sensory spots and the muscular scars. Pectinate fringes not visible with LM. Secondary pectinate fringes, cuticular ridges and apodemes (anteromesial thickenings of ventral pachycycli) absent.

Spinose processes and spines *Neocentrophyes intermedius* has hairy midlateral and middorsal spinose processes on segments 1–9 (Fig. 2b–d, f, j) and *N. satyai* on segments 1–10 (Fig. 3c, d, h, j). Both species with keel-shaped middorsal and midlateral processes, with an elongate base extending along most of the segment length. Sensory spots or intracuticular atria flanking the spinose processes are absent. *N. intermedius* with flexible and articulated middorsal spines on segments 10 and 11 (Fig. 2j). *N. satyai*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B
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C
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D
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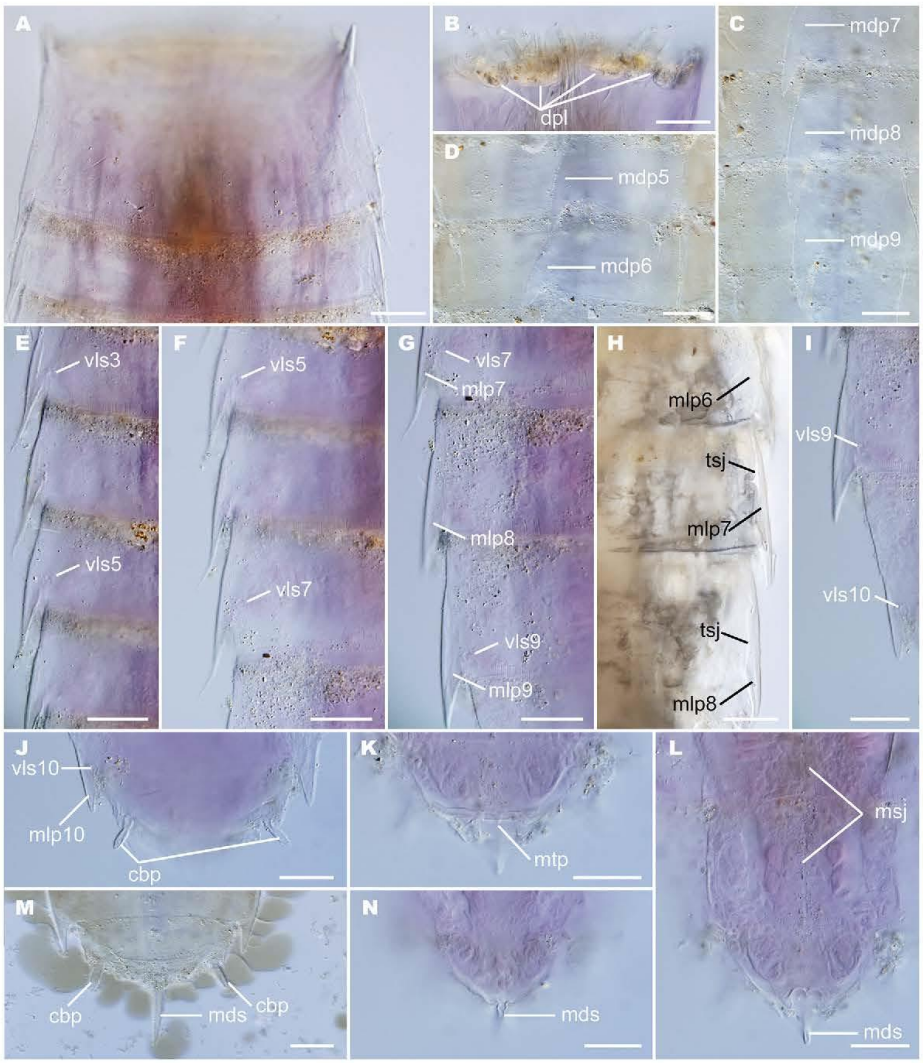


Fig. 3 *Neocentrophyes satyai.* Differential interference contrast photographs of female paratypes, except for **h**, showing female holotype. **a** Ventral view of segments 1–2. **b** Dorsal placids. **c** Detail showing middorsal processes on segments 7–9. **d** Detail showing middorsal processes on segments 5–6. **e** Ventral view of right sternal plates on segments 3–6. **f** Ventral view of right sternal plates on segments 5–7. **g** Ventral view of right sternal plates on segments 7–9. **h** Ventral view of left sternal plates on segments 6–8. **i** Ventral view of midlateral processes on segments 9–10. **j** Ventral view of segments 10–11. **k** Ventral view of segment 11. **l** Ventral view of segments 9–11. **m** Ventral view of segment 11. **n** Dorsal view of segment 11. *Scale bar* 20 µm. *cbp* cuticular bulbous protrusion, *dpl* dorsal placids, *mdp* middorsal process, *mds* middorsal spine, *mlp* midlateral process, *msj* midsternal junction, *mtp* midterminal process, *tsj* tergosternal junction, *vls* ventrolateral seta. Digits following labels refer to segment numbers

with an articulated and rigid middorsal spine on segment 11 instead of a middorsal spinose process (Fig. 3l–n). Both middorsal and midlateral spinose processes of segments 1–7 are more flexible (Figs. 2b–f, h–i, 3c, d) than those of segments 8 and 9 that appear much more robust and rigid and with acicular pointed tip (Figs. 2d, j–k, m, 3g, i, j). *N. intermedius* with two pairs of long and flexible penile spines, one attached to segment 10 and one to segment 11, both in paralateral positions (Fig. 2l, n). *N. intermedius* with a conspicuous midterminal process on segment 11,

Table 4 Measurements (μm) of female holotype and male paratype (ZMB 11517) of *Mixtophyes abyssalis* gen. et sp. nov

Character	Holotype ♀	Paratype ♂
TL	871	769
msw/TL (%)	24.3	27.7
lts/TL (%)	40.8	61.1
s1	131	121
s2	66	65
s3	67	76
s4	70	76
s5	78	77
s6	81	79
s7	88	82
s8	93	88
s9	98	96
s10	86	78
s11	47	47
sw1	205	210
sw2	197	209
sw3	202	210
sw4	208	209
sw5	210	210
sw6	211	211
sw7	212	213
sw8	205	209
sw9	192	203
sw10	167	180
sw11	146	456
lts	355	470
mds11	146	82

lts lateral terminal spine, *mds11* middorsal spine of segment 11, *msw*, maximum sternal width, *s1–s11* 1–11 trunk segments length, *sw1–sw11* sternal width of trunk segments 1–11; *TL* trunk length

Segment 2 Tergal plate with large middorsal and midlateral spinose processes similar to those on the preceding segment (Figs. 4a, b, 6i). Pairs of perispinal setae in paralateral positions appear to be present in both sexes (Fig. 4b). One pair of subdorsal and three pairs of laterodorsal sensory spots present (Fig. 6i). Pairs of laterodorsal muscular scars present between the two pairs of sensory spots located most laterally, on this and all following segments (Fig. 4b). Sternal plates with one pair of ventromedial sensory spots and muscular scars, the latter located mesially, adjacent to the sensory spots and almost in paraventral position (Fig. 4a).

Segment 3 Tergal plate similar to that of segment 2 (Fig. 6i), but without setae. One pair of subdorsal and two pairs of laterodorsal sensory spots present. The most mesial pair of laterodorsal sensory spots appears to be slightly laterally displaced (Figs. 4b, 6i). Sternal plates as on segment 2.

Segment 4 Tergal plate similar to that on segment 3, but with the laterodorsal pair of sensory spots located more mesially, aligned with those of segment 2. Sternal plates as those on segment 2, but with an additional pair of ventromedial sensory spots, located more laterally (Fig. 4a).

Segment 5 Tergal plate similar to that on segment 3 and sternal plates as those on segment 4, but with one pair of ventrolateral perispinal setae (Figs. 4a, 5c).

Segment 6 Tergal and sternal plates similar to those on segment 4 (Fig. 5c), but females with an additional pair of subdorsal sensory spots located close and anterior to the other subdorsal pair (Fig. 4b).

Segment 7 Tergal plate similar to that on segment 3, but with one pair of paralateral perispinal setae (Figs. 4b, 6j), and with a single paradorsal perispinal seta on the left side

Table 5 Summary of location of setae, sensory spots, spines and midterminal process in *Mixtophyes abyssalis* gen. et sp. nov. arranged by series

Segment	PD	SD	LD	PL	LV	VL	VM	MV
1		ss	ss, ss	ss		se(m), ss	ss	
2		ss	ss, ss, ss	se			ss	
3		ss	ss, ss				ss	
4		ss	ss, ss				ss, ss	
5		ss	ss, ss			se	ss, ss	
6		ss, ss(f)	ss, ss				ss, ss	
7	se*	ss	ss, ss	se			ss, ss	
8		ss, ss(f)	ss, ss	se			ss, ss	
9	se*	ss	ss, ss	se		se	ss, ss	
10		ss	ss, ss	ps(m)			ss, ss	
11		ss3			lts	msp	ss, ss, ss3	mtp(m)

f female condition of sexually dimorphic character, *LD* laterodorsal, *lts* lateral terminal spines, *LV* lateroventral, *m* male condition of sexually dimorphic character, *msp* minute spine of segment 11, *mtp* midterminal process, *MV* midventral, *PD* paradorsal, *PL* paralateral, *ps* penile spines, *SD* subdorsal, *se* setae, *ss* sensory spots type 1, *ss3* sensory spots type 3, *VL* ventrolateral, *VM* ventromedial

* marks that the seta is unpaired

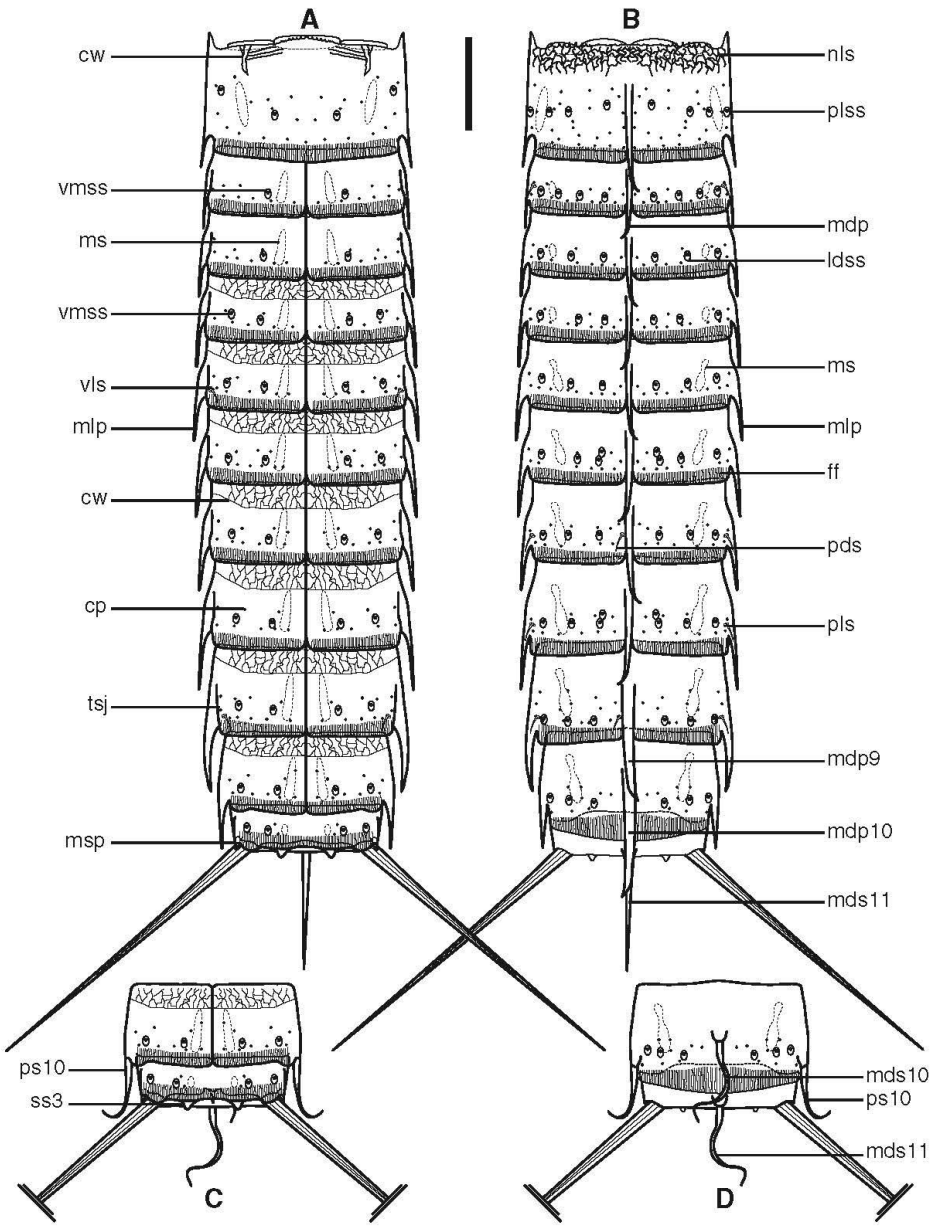


Fig. 4 Line art illustrations of *Mixtophyes abyssalis* gen. et sp. nov. **a** Female, ventral view. **b** Female, dorsal view. **c** Male, segments 10–11, ventral view. **d** Male, segments 10–11, dorsal view. *Scale bar* 100 µm. *cp* cuticular pore, *cw* cuticular wrinkles, *ff* free flap, *ldss* laterodorsal sensory spot, *mdp* middorsal process, *mds* middorsal spine, *mlp* midlateral process, *ms* muscular scar, *msp* minute spine of segment 11, *nls* net-like structure, *pds* paradorsal seta, *pls* paralateral seta, *plss* paralateral sensory spot, *ps* penile spine, *ss3* sensory spot type 3, *tsj* tergosternal junction, *vls* ventrolateral seta, *vmss* ventromedial sensory spot. Digits following labels refer to segment numbers

emerging from a notch in the middle region of the sternal plate (Fig. 2l). Midterminal process of *N. satyai* is shorter than that of *N. intermedius* (Fig. 3k). Segment 11 with one pair of small ventrolateral cuticular bulbous protrusions that are longer in *N. satyai* than in *N. intermedius* (Figs. 2l, 3j, m). The bulbous structures are articulated, with thick walls, a central canal and a blunt terminal end.

Setae Paradorsal perispinal setae present on segments 1–10 in *Neocentrophyes intermedius* (Fig. 2f, j) and at least on segments 1, 4–8 in *N. satyai*. The setae appear either single or in pairs in variable patterns, and their presence are very difficult to confirm in both cases because the middorsal processes usually fold over their position. Pairs of paradorsal perispinal setae were observed on segments 3, 5, 7 and 10 in different specimens of *N. intermedius*, but only those of segment 10 could be clearly observed in all animals. Paralateral perispinal setae were consistently found in both species on segments 1–10 and ventrolateral ones on segments 1, 3, 5–10 (Figs. 2a, e, h–k, m, 3e–g, i–j) (two specimens of *N. intermedius* and one of *N. satyai*—Fig. 3f- had no ventrolateral perispinal setae on segment 6).

Sensory spots Pairs of subdorsal and ventromedial type 3 sensory spots present on segment 11 only, with the ventromedial pair flanking the midterminal process. Remaining sensory spots throughout the trunk seem to belong to type 1 (but confirmation with SEM is required) and consist of one or two tufts of papillae. Distribution of sensory spots is summarized in Table 2 for *Neocentrophyes intermedius* and in Table 3 for *N. satyai*.

Genus *Mixtophyes* gen. nov.

Etymology

From Latin *mixtus*—mixed and Greek *phyes* (*φυής*)—characterized by a form, the commonly used suffix in homalorhagid genus names. The name refers to the mix of characters from *Neocentrophyes* and *Paracentrophyes* observed in the new genus.

Type species: *Mixtophyes abyssalis* sp. nov.

Diagnosis

Neocentrophyidae with a single sternal plate on the first trunk segment, without subdivision in its anterior region; pairs of rigid lateral terminal spines on segment 11 in both sexes; females with non-articulated middorsal spinose process on segment 10; males with a single pair of long and flexible penile spines located in paralateral position on segment 10.

Mixtophyes abyssalis sp. nov. (Figs. 4, 5, 6).

Fig. 5 *Mixtophyes abyssalis* gen. et sp. nov. SEM micrographs of paratypic male. **a** Ventral view. **b** Lateral view. **c** Ventral view of segments 5–6. **d** Ventral view of segment 9. **e** Ventral view of segments 10–11. **f** Ventral view of segment 1. **g** Mouth cone and outer oral styles. **h** Ventral view showing detail of anteriormost region of segment 1. **i** Base of midlateral process and paralateral seta on segment 9. **j** Lateral view of segments 7–11. **k** Detail showing ventromedial sensory spots on left sternal plate on segment 10. **l** Midlateral process of segment 9 in ventral view. **a**–**b** *Scale bar* 100 μm. **c**–**l** *Scale bar* 10 μm, except for **j**, 20 μm. *cw* cuticular wrinkles, *mdp* middorsal process, *mds* middorsal spine, *mlp* midlateral process, *oos* outer oral styles, *pds* paradorsal seta, *pls* paralateral seta, *po* protonephridial opening, *ps* penile spine, *ss3* sensory spot type 3, *tsj* tergosternal junction, *vls* ventrolateral seta. Sensory spots are marked with *circlets*. Digits following labels refer to segment numbers

Etymology

From Latin *abyssus*, abyss, deep-sea; referring to the sampling localities at 5,136–5,174 m depth in the Atlantic Ocean where the new species was found.

Type material

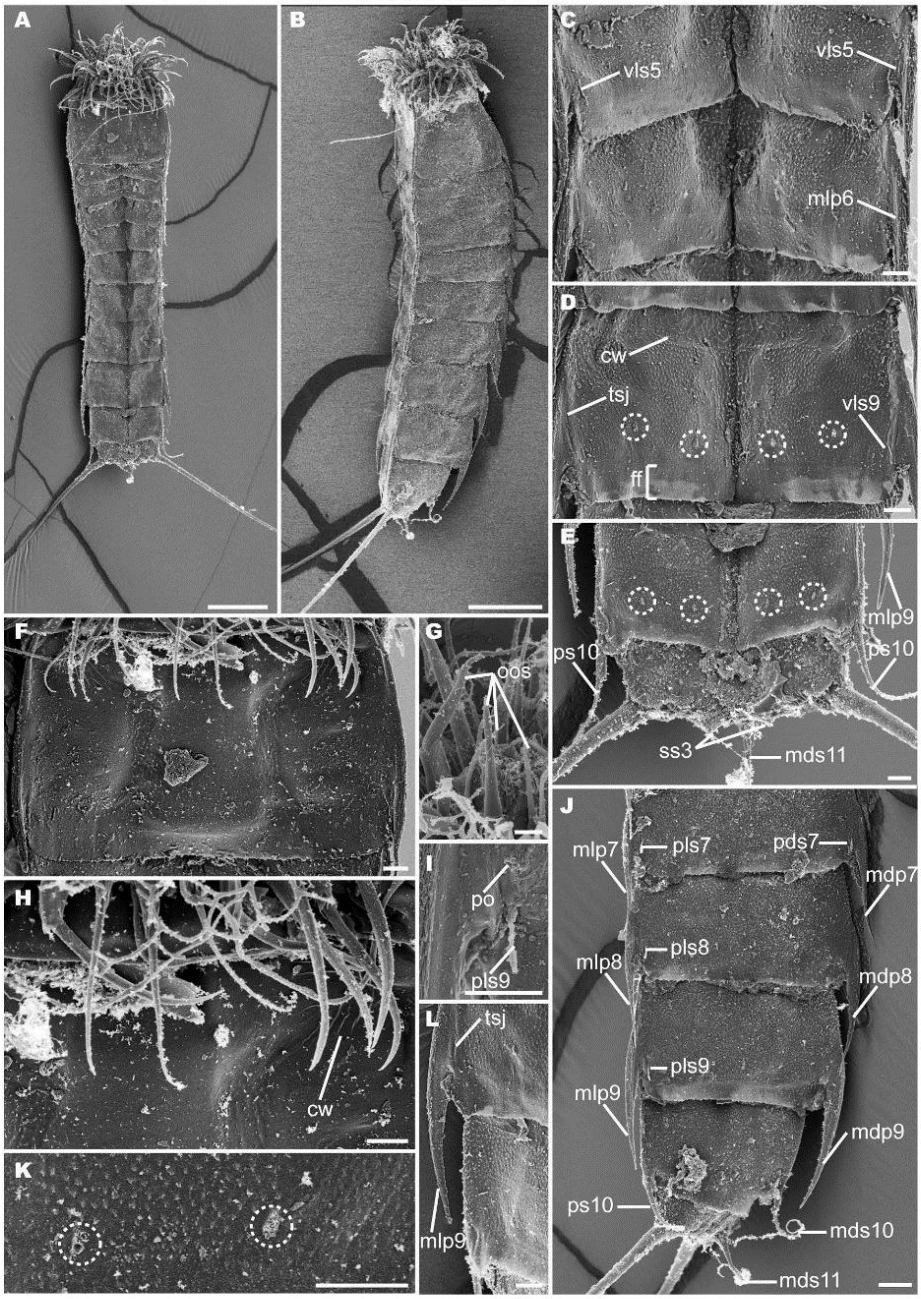
Holotype, adult female, collected on March 23, 2005, in the Guinea Basin at DIVA2 Station M63/2 98, position: 00°37′12″N, 006°28′06″W (Fig. 1), from mud at 5,165–5,174 m depth, mounted in Fluoromount G®, stored at the MfN under accession number: ZMB 11516. Two paratypes, adult males: one collected on March 19, 2005, in the Guinea Basin at DIVA2 Station M63/2 79, position: 00°50′00″N, 005°35′00″W (Fig. 1), from mud at 5,136–5,142 m depth, mounted in Fluoromount G®, and stored at the MfN under accession number: ZMB 11517; and the other one collected at same date and locality as allotype, mounted for SEM, stored at the MfN under accession number: ZMB 11518.

Diagnosis

Same as genus diagnosis combined with presence of large middorsal spinose processes on segments 1–9 with similar size from segment 1 to 6 and increasing in length toward the posterior trunk segments; single (left) paradorsal perispinal seta on segments 7 and 9, pairs of paralateral setae on segments 2, 7–9 and ventrolateral setae on segments 1 (in males only), 5 and 9; pairs of very minute ventrolateral spines on segment 11; cuticular ornamentation of the anterior margin of first trunk segment as wrinkles, dorsally and ventrolaterally; type 1 sensory spots consisting of one or two tufts of papillae around a central pore.

Description

All dimensions and measurements of the examined specimens are summarized in Table 4 and distribution of sensory spots and setae in Table 5.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C
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pls7
pds7
mdp7
mlp8
pls8
mdp8
pls9
mlp9
mdp9
ps10
mds10
mds11

Mouth cone Cuticular surface of mouth cone with longitudinal wrinkles, and nine outer oral styles attached distally. Two kinds of outer oral styles: five large and well-developed styles consisting of two articulating units, alternating with four smaller, non-articulated ones.

Neck Four dorsal and three ventral placids; ventrolateral ones appear soft and inconspicuous in LM. Remaining placids are more robust, with concave surfaces. The two medial dorsal placids are slightly wider than the more lateral ones. The rectangular midventral placid is broad, up to 1/3 of the segment width (Fig. 6g).

Trunk Eleven segments in trunk (Figs. 4a, b, 5a, b, 6a, b), with the first one consisting of one tergal and one sternal plate, without any complete or partial subdivision in the anterior region of the sternal plate (Figs. 4a, 5a, f, h, 6h). Segments 2–10 with one tergal and two sternal plates and segment 11 with one tergal and a single sternal plate (Figs. 4a, c, 6a, k). Sternal plates are flattened, whereas the tergal plate is vaulted, giving the trunk a triangular shape in cross-section. The segment width is rather constant along the trunk, reaching its maximum sternal width at segment 7 and tapering progressively from this point toward the terminal segment. Tergosternal junctions are clearly observed on segments 1–9 and 11, whereas they could not be identified on segment 10 (Figs. 4a, c, 5d, l, 6f). Markings of the tergosternal junctions are not equally developed along the segments, and they are easily observed at the bases of the midlateral spinose processes, whereas they are inconspicuous in the anteriormost parts of the segments (except for segment 1). Segments 1–9 with middorsal spinose processes pointing backward and extending over the following segment. The middorsal spinose processes are of similar size on segments 1–6 and then increase progressively in length toward the posterior segments 7–9 (Figs. 4b, 5b, j, 6b). Males with articulated, flexible and soft middorsal spines on segments 10 and 11 (Figs. 4d, 5j); females with one non-articulated, rigid and robust middorsal spinose process on segment 10 and one articulated, robust middorsal spine on segment 11 (Figs. 4b, 6b, e). Neither intracuticular atria nor paradorsal sensory spots are present at any segment (Fig. 6b). Hairy midlateral spinose processes with acicular pointed tips are present on segments 1–9 in males and 1–10 in females (Figs. 4a–c, 5a, b, 6a, b). Trunk surface with numerous cuticular pores (Figs. 4a–d, 6c, i) and scale-like cuticular hairs, the latter being absent around the sensory spots and muscular scars (Figs. 5k, 6i). Segments 4–10 with cuticular wrinkles in the anteriormost region of the sternal plates (Figs. 4a, c, 5d). Most segments with two pairs of muscular scars, located in laterodorsal (segments 1–10) and ventromedial (segments 1–11) positions (Fig. 4a–d); ventral muscular scars on segment 1 located more lateral than those of the remaining segments. The laterodorsal scars are large, elongate and oval on segment 1, smaller and more rounded on segments 2–4 and tenpin shaped on segments 5–10, whereas the ventromedial ones are elongate (Fig. 4a–d). Free flaps of tergal and sternal plates are striated and well developed, overlapping a broad portion of the anterior area of the following segment (Figs. 4a, b, 5d, 6f, i). Inconspicuous pectinate fringes are present on dorsal and ventral sides, visible with SEM only. Secondary pectinate fringes, cuticular ridges and apodemes absent. Pachycycli and peg and socket joints not well developed (Fig. 6a, b).

Fig. 6 *Mixtophyes abyssalis* gen. et sp. nov. Differential interference contrast photographs. **a–c**, **e–k** Holotypic female. **d** Paratypic male (ZMB 11517). **a** Ventral view. **b** Dorsal view. **c** Dorsal view of left half of segment 1. **d** Dorsal view of segments 8–9. **e** Middorsal process and spines on segments 10–11. **f** Right sternal plates on segments 9–10. **g** Ventral placids. **h** Ventral view of segment 1. **i** Dorsal view of left half of segments 2–3. **j** Midlateral process of segment 7. **k** Ventral view of segments 10–11. *Scale bar* 20 µm, except for **d**, 50 µm. *cp* cuticular pore, *cw* cuticular wrinkles, *ff* free flap, *mdp* middorsal process, *mds* middorsal spine, *mlp* midlateral process, *msp* minute spine of segment 11, *nls* net-like structure, *pls* paralateral seta, *ss3* sensory spot type 3, *tsj* tergosternal junction, *vpl* ventral placid. Sensory spots are marked with circlets, except on D, where the circlet marks the gonad with sperm. Digits following labels refer to segment numbers

Segment 1 Anterolateral margins of tergal plate projecting into horn-like extensions (Fig. 4a, b). Anterior edge of tergal plate strongly denticulated, followed by a band of cuticular wrinkles forming a reticulate net-like ornamentation all along the anterior margin (Figs. 4b, 6c). Middorsal spinose process with an elongate base and a flexible terminal end (Figs. 5b, 6c). This conspicuous, keel-shaped structure begins at the anterior third of the segment and surpasses half of the following segment. A longitudinal band of short cuticular hairs stretches along the basal part of the process. Sensory spots on the tergal plate include one subdorsal, two laterodorsal and one paralateral pairs (Figs. 4b, 6c). Sensory spots on this and all following segments belong to type 1 and consist of numerous small papillae located anterior to one or two tufts of 8–10 larger cuticular papillae. Each tuft of 8–10 papillae is arranged around a central pore (Fig. 5k). No cilia have been observed emerging from the central pores. Ventral side with a single sternal plate, neither complete nor partial subdivision (Figs. 4a, 5a, f, h, 6h). Anterior edge of sternal plate denticulated, similar to that of the tergal plate. Anterolateral area of sternal plate with conspicuous transverse and longitudinal cuticular wrinkles; central anterior area smooth (Figs. 4a, 5f, h, 6g, h). Midlateral spinose processes covered by short hairs and flanked by ventrolateral perispinal setae in males (absent in females). Pairs of ventromedial and ventrolateral sensory spots present, the latter located more anteriorly. Scale-like cuticular hairs cover the posterior half of the segment (Fig. 5f). Deep, rounded notches appear at the free flap on both sides of the middorsal and midlateral spinose processes (Figs. 4a, b, 5f).

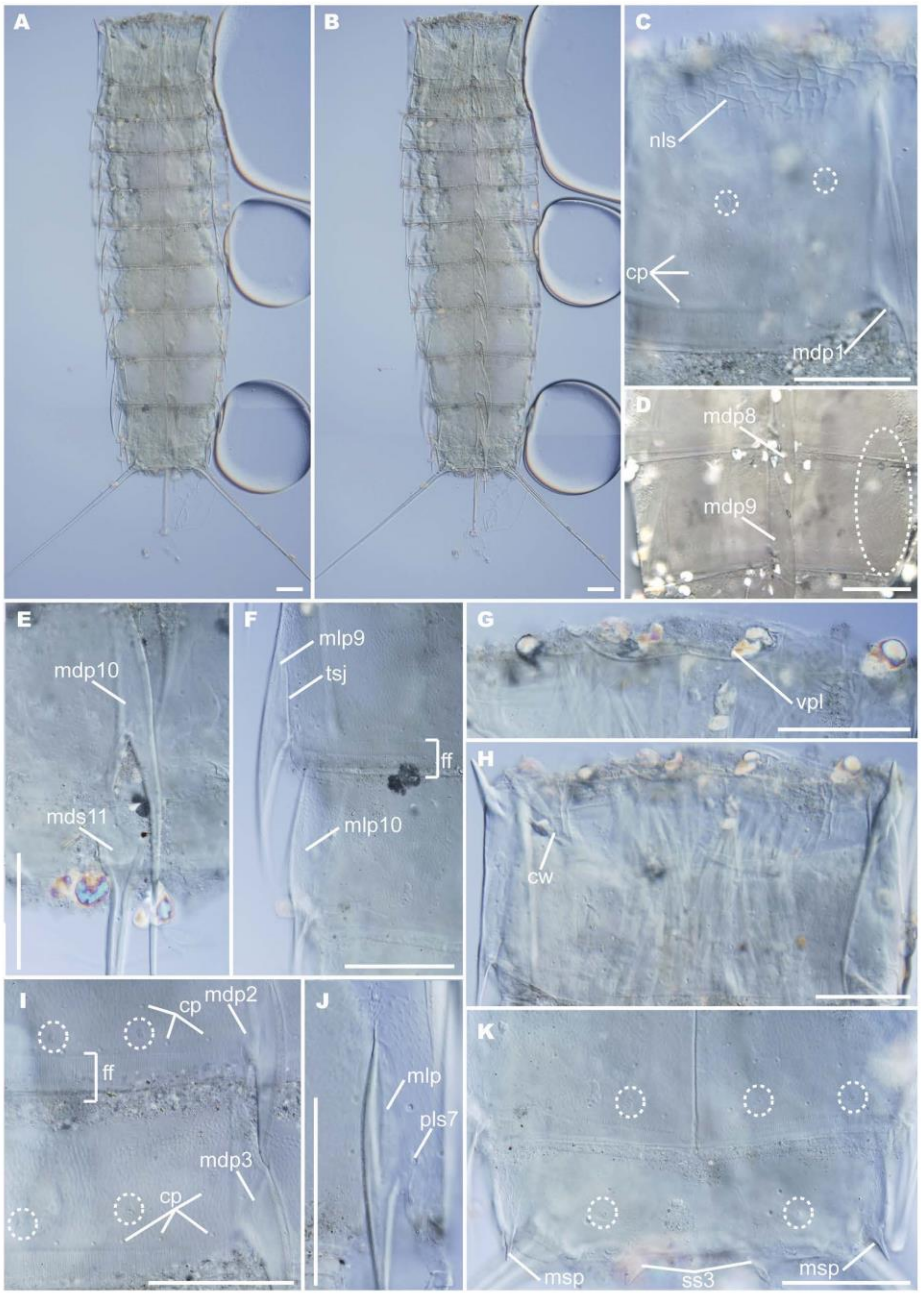
A
B
C
nls
cp
mdp1
D
mdp8
mdp9
E
mdp10
mds11
F
mlp9
tsj
ff
mlp10
G
vpl
H
cw
I
cp
mdp2
ff
mdp3
cp
J
mlp
pls7
K
msp
ss3
msp

Table 4 Measurements (μm) of female holotype and male paratype (ZMB 11517) of *Mixtophyes abyssalis* gen. et sp. nov

Character	Holotype ♀	Paratype ♂
TL	871	769
msw/TL (%)	24.3	27.7
lts/TL (%)	40.8	61.1
s1	131	121
s2	66	65
s3	67	76
s4	70	76
s5	78	77
s6	81	79
s7	88	82
s8	93	88
s9	98	96
s10	86	78
s11	47	47
sw1	205	210
sw2	197	209
sw3	202	210
sw4	208	209
sw5	210	210
sw6	211	211
sw7	212	213
sw8	205	209
sw9	192	203
sw10	167	180
sw11	146	456
lts	355	470
mds11	146	82

lts lateral terminal spine, *mds11* middorsal spine of segment 11, *msw*, maximum sternal width, *s1–s11* 1–11 trunk segments length, *sw1–sw11* sternal width of trunk segments 1–11; *TL* trunk length

Segment 2 Tergal plate with large middorsal and midlateral spinose processes similar to those on the preceding segment (Figs. 4a, b, 6i). Pairs of perispinal setae in paralateral positions appear to be present in both sexes (Fig. 4b). One pair of subdorsal and three pairs of laterodorsal sensory spots present (Fig. 6i). Pairs of laterodorsal muscular scars present between the two pairs of sensory spots located most laterally, on this and all following segments (Fig. 4b). Sternal plates with one pair of ventromedial sensory spots and muscular scars, the latter located mesially, adjacent to the sensory spots and almost in paraventral position (Fig. 4a).

Segment 3 Tergal plate similar to that of segment 2 (Fig. 6i), but without setae. One pair of subdorsal and two pairs of laterodorsal sensory spots present. The most mesial pair of laterodorsal sensory spots appears to be slightly laterally displaced (Figs. 4b, 6i). Sternal plates as on segment 2.

Segment 4 Tergal plate similar to that on segment 3, but with the laterodorsal pair of sensory spots located more mesially, aligned with those of segment 2. Sternal plates as those on segment 2, but with an additional pair of ventromedial sensory spots, located more laterally (Fig. 4a).

Segment 5 Tergal plate similar to that on segment 3 and sternal plates as those on segment 4, but with one pair of ventrolateral perispinal setae (Figs. 4a, 5c).

Segment 6 Tergal and sternal plates similar to those on segment 4 (Fig. 5c), but females with an additional pair of subdorsal sensory spots located close and anterior to the other subdorsal pair (Fig. 4b).

Segment 7 Tergal plate similar to that on segment 3, but with one pair of paralateral perispinal setae (Figs. 4b, 6j), and with a single paradorsal perispinal seta on the left side

Table 5 Summary of location of setae, sensory spots, spines and midterminal process in *Mixtophyes abyssalis* gen. et sp. nov. arranged by series

Segment	PD	SD	LD	PL	LV	VL	VM	MV
1		ss	ss, ss	ss		se(m), ss	ss	
2		ss	ss, ss, ss	se			ss	
3		ss	ss, ss				ss	
4		ss	ss, ss				ss, ss	
5		ss	ss, ss			se	ss, ss	
6		ss, ss(f)	ss, ss				ss, ss	
7	se*	ss	ss, ss	se			ss, ss	
8		ss, ss(f)	ss, ss	se			ss, ss	
9	se*	ss	ss, ss	se		se	ss, ss	
10		ss	ss, ss	ps(m)			ss, ss	
11		ss3			lts	msp	ss, ss, ss3	mtp(m)

f female condition of sexually dimorphic character, *LD* laterodorsal, *lts* lateral terminal spines, *LV* lateroventral, *m* male condition of sexually dimorphic character, *msp* minute spine of segment 11, *mtp* midterminal process, *MV* midventral, *PD* paradorsal, *PL* paralateral, *ps* penile spines, *SD* subdorsal, *se* setae, *ss* sensory spots type 1, *ss3* sensory spots type 3, *VL* ventrolateral, *VM* ventromedial

* marks that the seta is unpaired

of the middorsal process. Middorsal and midlateral spinose processes more elongated, reaching the posterior third of the following segment (Figs. 5a, b, j, 6b). Sternal plates as on segment 4.

Segment 8 Tergal and sternal plates similar to those on segment 6, but with one pair of paralateral perispinal setae (Fig. 4b). Middorsal and midlateral spinose processes with thicker bases (Figs. 5a, b, j, 6b).

Segment 9 Tergal plate similar to that on segment 7, but with larger middorsal and midlateral spinose processes; both structures with rigid tips, contrary to the more flexible ones of the preceding segments (Figs. 5j, l, 6a, b, f). Paralateral protonephridial opening surrounded by several minute hairs, not sieve-like (Fig. 5i). Sternal plates similar to those on segment 4, but with one pair of ventrolateral perispinal setae (Fig. 5d).

Segment 10 Males with a flexible and soft middorsal spine (articulated) departing from the medial portion of the segment (Figs. 4d, 5j) and extending far beyond the posterior end of the trunk (Fig. 5c, d). Posterior dorsal margin of the segment straight in males, not interrupted by notches in subdorsal position (Fig. 5j). Furthermore, males without midlateral spinose processes, but with one pair of long and flexible paralateral penile spines (Figs. 4c, d, 5e, j). Females with a robust, rigid and pointed middorsal spinose process (non-articulated), similar to that characterizing the preceding segment (Figs. 4b, 6e), and extending far beyond the posterior end of the trunk segment (Figs. 4a, b, 6a, b). Midlateral spinose processes shorter than the middorsal one (Figs. 4a, b, 6a, b). Posterior dorsal margin in females with notches at both sides of the middorsal spinose process. Both sexes with three pairs of sensory spots present, one in subdorsal and two in laterodorsal positions. Sternal plates with two pairs of ventromedial sensory spots (Figs. 5e, k, 6k), both located in the same position as those on the preceding segments. Posterior ventral margin of the segment straight, but with extensions in the ventrolateral areas (Figs. 5e, 6k).

Segment 11 One tergal and a single sternal plate (Figs. 4a, c, 6k). Both sexes with middorsal spines (articulated) and one pair of robust, lateral terminal spines. Midlateral spinose processes not present. The middorsal spine of males is similar to that on segment 10 (Figs. 4c, d, 5e, j). Contrarily, the middorsal spine of females appears more like the middorsal process of segment 10, being robust, rigid and pointed (Fig. 6b). However, its internal structure is remarkably different. It is not constituted by a single piece, but appears articulated, divided into two parts, and attaches to the posterior portion of the segment (Fig. 6e). Males without penile spines on this segment (Figs. 4c,d, 5e, j), and with a tiny midterminal process, emerging from a notch in the medial region of the plate (Fig. 6k). Both sexes with two pairs of ventromedial sensory spots on sternal plates (Fig. 6k). Type 3 sensory spots present in subdorsal and ventromedial positions, protruding from the posterior margin of the segment (Figs. 5e, 6k). One pair of very minute, conical ventrolateral spines present (Figs. 4a, c, 6k). The posterior margin of the segment is straight along the dorsal side, whereas the ventral one has two extensions over the lateral terminal spines, partly covering the minute ventrolateral spines (Figs. 5e, 6k).

Summary of sexually dimorphic characters

Males with a flexible middorsal spine (articulated) on segment 10; females with middorsal spinose process (non-articulated) instead. Middorsal spine (articulated) on segment 11 present in both sexes, rigid in females and flexible and shorter in males. Males without midlateral spinose processes on segment 10; females with well-developed and rigid spinose process. Females with one additional pair of subdorsal sensory spots on segments 6 and 8. Furthermore, males characterized by one pair of paralateral penile spines on segment 10, midterminal process and one pair of ventrolateral perispinal setae on segment 1.

Associated kinorhynch fauna

Several other kinorhynch species co-occurred with *M. abyssalis* gen. et sp. nov. Station M63/2 79 revealed three conspecific specimens (two adults and one juvenile) that could not be identified to genus level. These organisms resemble both *Antygomonas* and *Sphenoderes*; further studies are needed for a detailed description. The sampled sediment also contained juveniles and adults of a new species of *Echinoderes* with middorsal spines on segments 4–8, lateroventral spines/tubes on segments 5–10, dorsolateral spines on segment 2 and lateral accessory tubes on segment 8. Station M63/2 98 contained several specimens of *Echinoderes* spp. and two new species of *Pycnophyes*.

Discussion

Re-examination of *Neocentrophyes intermedius and N. satyai*

Re-examination of the type series of the two known *Neocentrophyes* species revealed novel information about their morphology, and it also enabled us to correct some details in the original description (Higgins 1969). First of all, the examination revealed new details about the cuticular ornamentation of segment 1, information on the sensory spot distribution along the trunks of the two species, together with the absence of apodemes, cuticular ridges and secondary pectinate fringes along the segments.

Nevertheless, the re-examination confirmed traits reported in the original description, while revealing some shortcomings, some of which being rather important. Most importantly, it showed that the sternal plate of the terminal segment is undivided and hence not differentiated into two sternal plates as originally reported (Higgins 1969). This particular character could be of interest in a comparative aspect, since it varies between the homalorhagid genera. Among species of *Pycnophyes* and *Kinorhynchus*, segment 11 is consistently through the genera composed of one tergal and two sternal plates (e.g., Higgins 1983; Adrianov and Malakhov 1999; Sánchez et al. 2011, 2014), whereas the three known species of *Paracentrophyes* and *Mixtophyes abyssalis* gen. et sp. nov. have a single sternal plate in the terminal segment (Higgins 1983; Sørensen et al. 2010; present contribution).

Additional details differing from the original description by Higgins (1969) include: (a) the position of the penile spines, the first pair being placed on segment 10 and the second pair on segment 11, and not both pairs located on segment 11; (b) the occurrence of a middorsal, basally articulating spine, and not a spinose process on segment 11 in females; (c) the absence of ventrolateral setae on segment 4 (originally reported as present). The latter may seem to be an irrelevant detail, but since distribution of setae appears to be a diagnostic character among homalorhagid species, this information would be important in the future if new species of *Neocentrophyes* were found.

Based on the added and corrected information, emended diagnoses for Neocentrophyidae and *Neocentrophyes* are included below.

Originally, *Neocentrophyes intermedius* and *N. satyai* were described as two distinct species by Higgins (1969), but later Higgins (1983) considers whether the two entities in fact could represent male and female of the same species. This possibility appears very likely, as the two species are mainly distinguished by characters that usually relate to sexual dimorphism. However, the geographic distance between the species' type localities, Madagascar for *N. intermedius* and Bay of Bengal for *N. satyai* obviously argues against this hypothesis, but then again, India and Madagascar were actually connected and part of Gondwana, and their tectonic plates did not separate before late Cretaceous, about 90 mya. Of course, one would expect that 90 million years' genetic separation of the Madagascan and Indian populations would have result in separate speciation at some point, but at least a paleohistorical connection between the two species or populations exists. Currently, it is impossible to determine whether *N. intermedius* and *N. satyai* are conspecific or not, hence new sampling near the species' type localities in order to collect male *N. satyai* and female *N. intermedius* would be required to answer the question.

Diagnostic characters in *Mixtophyes abyssalis* gen. et sp. nov

Mixtophyes abyssalis gen. et sp. nov. is obviously closely related to the homalorhagid genera *Pycnophyes, Kinorhynchus, Paracentrophyes* and *Neocentrophyes* and displays several typical homalorhagid traits, such as the almost rectangular outline of the trunk, the arrangement of the placids and the general absence of spines on segments 1–9. The new genus and species is, however, also easily distinguished from species of *Kinorhynchus* and *Neocentrophyes* by the presence of lateral terminal spines, which are lacking in species of the two mentioned genera (Zelinka 1928; Sheremetevskij 1974; Higgins 1969). It is also distinguished from species of *Pycnophyes* by its composition of the sternal plate on segment 1, being formed from a single plate and without traces of a subdivision, whereas the sternal plate is subdivided into one midsternal and two episternal plates in *Pycnophyes* (see, e.g., Higgins 1983; Sánchez et al. 2011, 2014). Perhaps *M. abyssalis* gen. et sp. nov. shows the closest resemblance to the species of *Paracentrophyes*, as they also have lateral terminal spines and an undivided sternal plate on segment 11. However, species of *Paracentrophyes* are characterized by having a sternal plate on segment 1 with partially developed lines, marking an incomplete differentiation into epi- and midsternal plates (Higgins 1983; Sørensen et al. 2010), whereas *M. abyssalis* gen. et sp. nov. has no markings at all on the sternal plate of segment 1.

Hence, the combination of a single sternal plate on segment 1 without partial subdivision, and lateral terminal spines, makes *M. abyssalis* gen. et sp. nov. unique among homalorhagids.

Notes on selected morphological characters in *M. abyssalis* gen. et sp. nov.

Besides the composition of segment 1, some additional characters deserve a special attention in *M. abyssalis* gen. et sp. nov. The cuticle generally appears rather thin, and pachycycli and peg and socket joints are very indistinct. This is also common for other species of Neocentrophyidae (see e.g., Higgins 1969, 1983), whereas these structures appear much more well developed in species of Pycnophyidae (Kristensen and Higgins 1991). The presence of keel-shaped middorsal and midlateral spinose processes, with flexible posterior ends, is also shared with all other species of Neocentrophyidae (Zelinka 1928; Higgins 1969, 1983; Sørensen et al. 2010), whereas adult individuals of species of Pycnophyidae do not have midlateral processes and the middorsal ones are never flexible (Sánchez et al. 2011). Most sensory spots of the new genus and species belong to type 1 (see Nebelsick 1992), mainly consisting of

one or two tufts of 8–10 cuticular papillae, each arranged around a central pore. Similar sensory spots have been found in all species of Neocentrophyidae (Higgins 1983; Neuhaus 1995; Sørensen et al. 2010; present contribution) but only in two species of Pycnophyidae (Adrianov and Malakhov 1994; Sánchez et al. 2014). Also the secondary pectinate fringes are lacking in *Mixtophyes abyssalis* gen. et sp. nov., which again is a trait shared with species of *Neocentrophyes* and *Paracentrophyes* (Higgins 1969, 1983; Sørensen et al. 2010; present contribution). Contrarily, most *Pycnophyes* and *Kinorhynchus* species have one or several secondary pectinate fringes that usually appear very conspicuous at SEM (Higgins 1983; Sánchez et al. 2011, 2013, 2014).

Sexual dimorphism in species of *Pycnophyes* and *Kinorhynchus* is usually expressed through the presence of two or three pairs of penile spines on the last trunk segment and one pair of large ventral tubes on segment 2 (supposedly, only five out of 54 species of *Pycnophyes* species lack these structures, see Neuhaus 2013). Contrarily, males of *Neocentrophyes* and *Paracentrophyes* always bear only two pairs of flexible and long penile spines, one on segment 10 and one on segment 11, and lack the large ventral tubes on segment 2 (Higgins 1969, 1983; Kristensen and Higgins 1991, Sørensen et al. 2010). Males of *Mixtophyes abyssalis* gen. et sp. nov. have only a single pair of flexible and long penile spines, located on segment 10, and the ventral tubes on segment 2 are missing. Males of *Paracentrophyes*, *Neocentrophyes* and *Mixtophyes* gen. nov. share the presence of soft and flexible middorsal spines on segments 10 and 11. However, females of *Paracentrophyes* have rigid middorsal spines on segments 10 and 11, whereas females of *Neocentrophyes* and *Mixtophyes* gen. nov. have rigid middorsal spine (articulated) on segment 11 only, and instead a middorsal process (hence, non-articulated) on segment 10 (Higgins 1969, 1983; Sørensen et al. 2010).

Phylogenetic position and taxonomic status of the new taxon

Kinorhynch phylogeny, and hence also homalorhagid phylogeny, was recently addressed in two studies based on molecular sequence data from 18S rRNA (Dal Zotto et al. 2013) and combined 18S rRNA and 28S rRNA (Yamasaki et al. 2013). The results of the two studies are congruent in several ways, e.g., they confirm a close relationship between species of *Pycnophyes* and *Kinorhynchus* and they indicate that *Dracoderes* is a homalorhagid taxon as well. Dal Zotto et al. (2013) also suggest that the aberrant genus *Franciscideres* (and perhaps even *Cateria*) is more closely related to the homalorhagids or it could be a very basal homalorhagid or even a basal kinorhynch. However, none of the two studies were able to include sequence data from a representative of *Neocentrophyes*, and even though data were included for *Paracentrophyes anurus* Sørensen et al., 2010, it was not possible for any of the studies to clarify its exact position inside Homalorhagida.

More information about the phylogenetic position of these two particular genera would have been desirable, since *Mixtophyes abyssalis* gen. et sp. nov.—based on the characters discussed above—shows some affinities to both of them. However, the results from Yamasaki et al. (2013) and Dal Zotto et al. (2013) can still be helpful and with a few assumptions, based on the observed morphological traits, it would be possible to establish a tentative position of *Mixtophyes* gen. nov. until more data become available.

First, the typical overall appearance of the four original homalorhagid taxa, i.e., the stout, rectangular trunk with almost parallel sides; represents a derived condition, opposed to the more spindle-shaped trunk, found in *Dracoderes*, *Franciscideres* and many cyclorhagids as well as in loriciferans, taken as an outgroup. This would suggest that *Pycnophyes*, *Kinorhynchus*, *Paracentrophyes*, *Neocentrophyes* and *Mixtophyes* gen. nov. represent a monophyletic clade. The results of the phylogenetic analyses made by Yamasaki et al. (2013) and Dal Zotto et al. (2013) are compatible with the idea that the condition of segment 1 in *Pycnophyes* and *Kinorhynchus*, with one tergal, two episternal and a midsternal plate, also represents a derived condition. Hence, it makes sense to imagine an evolutionary character transition going from a segment 1 formed from a closed ring, as expressed in *Dracoderes* and *Franciscideres* as well as in cyclorhagids (outgroup), through differentiation into a single tergal and sternal plate, as expressed in *Neocentrophyes*, *Mixtophyes* gen. nov. and, to some extent, *Paracentrophyes*, and a final differentiation of the sternal plate into one midsternal and two episternal plates. If this hypothetical transition series is correct, it seems likely that the condition of the sternal plate of segment 1 in *Paracentrophyes*, with a partially developed subdivision of the plate represents an intermediate stage between the condition in, on the one hand, *Neocentrophyes* and *Mixtophyes* gen. nov., and, on the other hand, *Pycnophyes* and *Kinorhynchus*. This would suggest that *Paracentrophyes* represents the sister taxon to *Pycnophyes* and *Kinorhynchus* and that *Neocentrophyes* and *Mixtophyes* gen. nov. branch off more basally (Fig. 7a–c).

This proposed character transition is also supported by ontogeny. The postembryonic development of *Pycnophyes dentatus* (Reinhard, 1881), *P. kielensis* Zelinka, 1928 and *Paracentrophyes praedictus* Higgins, 1983 is known in detail. In juvenile stages of these three species, segment 1 consists of a tergal plate and a single, undivided sternal plate (Neuhaus 1993, 1995). The partial or total differentiation of the sternal plate into three is observed only in the adult stage in *Pycnophyes* (Neuhaus 1993), whereas in

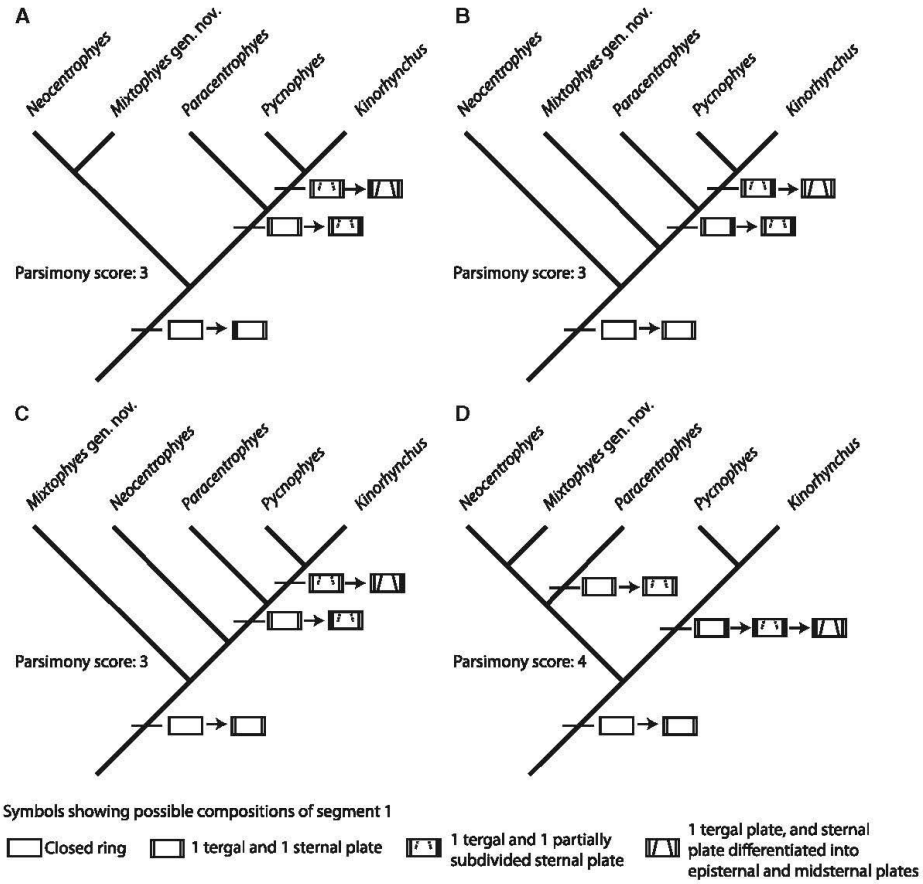

Fig. 7 Four different topologies with the evolutionary plate transformations on segment 1, optimized in three most parsimonious (**a**–**c**) and one less parsimonious (**d**) ways. The putative transformation series goes through following stages: (1) segment composed of a closed ring (putative ground pattern), (2) segment composed of one tergal and one sternal plate, (3) segment composed of one tergal plate and a partially differentiated sternal plate, (4) segment composed of one tergal plate and sternal plate differentiated into one midsternal and two episternal plates

Paracentrophyes, it usually appears in the adult stage (Neuhaus 1995) even though it may also occur in the J-6 stage (Sánchez, pers. obs.). This also indicates that the undivided sternal plate would be plesiomorphic condition relative to the subdivided plate.

The proposed scenario seems likely, but it is somehow obscured by other two traits: the presence of lateral terminal spines and the condition of segment 11. This segment consists of a tergal and paired sternal plates in *Pycnophyes* and *Kinorhynchus* (Zelinka 1928; Sheremetevskij 1974), and a tergal and an unpaired sternal plate in *Neocentrophyes*, *Mixtophyes* gen. nov. and *Paracentrophyes* (Higgins 1969, 1983; Sørensen et al. 2010; present contribution). This character alone would agree with a monophyletic clade formed by the three latter taxa (Fig. 7d), but it would also contradict the scenario proposed above (Fig. 7a–c). It is not possible to optimize both characters on the same tree (i.e., the condition of the sternal plates on segments 1 and 11) without making at least one of them homoplastic. Under these conditions, we would consider the transformation series of segment 1 as the one that provides most significant phylogenetic information, since we know that the plate composition of segment 11 may vary, even at intrageneric levels (see Neuhaus and Blasche 2006; Sørensen et al. 2013). Lateral terminal spines are present in species of all kinorhynch genera

except those of *Kinorhynchus* and *Neocentrophyes* (Zelinka 1928; Sheremetevskij 1974; Higgins 1969; Sørensen and Pardos 2008). However, since no other character points toward a sister-group relationship between these two genera, we would consider the loss of lateral terminal spines as two independent autapomorphic traits and therefore without phylogenetic relevance above genus level.

Conclusively, since the presence of a single, undivided sternal plate on segment 1 would represent a plesiomorphic condition within a clade with *Mixtophyes* gen. nov. and the four traditional homalorhagid genera, its presence in *Neocentrophyes* and *Mixtophyes* gen. nov. cannot be used as an argument to consider the two genera as closest relatives—or at least overrule other equally parsimonious alternatives. Likewise, the partial differentiation of the sternal plate on segment 1 in species of *Paracentrophyes* would suggest that this genus is closer to Pycnophyidae. Hence, and after disregarding plate arrangement in segment 11 and presence of lateral terminal spines as suitable characters, the plate configuration of segment 1 suggests that *Mixtophyes* gen. nov. is either sister taxon to *Neocentrophyes* (Fig. 7a), to an assemblage of *Paracentrophyes*, *Pycnophyes*, and *Kinorhynchus* (Fig. 7b) or to a clade with the four mentioned genera (Fig. 7c). As a consequence, the monophyly of Neocentrophyidae also has to be questioned, since the hypothetical transformation series of segment 1 suggests that *Paracentrophyes* is closer to *Pycnophyes* and *Kinorhynchus*. However, as phylogenetic studies on kinorhynch relationships currently are being carried out by the authors of this paper and several collaborators, we prefer to stick with the traditional classification for now and wait with eventual revisions until the results of the phylogenetic analyses are available.

Neocentrophyidae Higgins 1969—emended diagnosis

Homalorhagida with sternal plate on first trunk segment undivided or with partial divisions at the anteriormost region of the plate; remaining trunk segments with one tergal and two sternal plates, except for the terminal segment, consisting of a tergal and a single sternal plate. Articulated rigid lateral terminal spines on segment 11 present or absent; non-articulated middorsal spinose processes on segments 1–9 in both sexes; females with non-articulated middorsal spinose process also on segment 10 or with an articulated, pointed and rigid middorsal spine instead; males always with an articulated, flexible and soft middorsal spine on segment 10; articulated middorsal spine on segment 11 in both sexes, rigid in females and flexible in males; non-articulated midlateral spinose processes on segments 1–9; processes may be absent or present on segments 10 and 11; males with one pair of long and flexible penile spines on segment 10; additional pair of similar penile spines on segment 11 present or absent; pachycycli, peg and socket joints, and apodemes (anteromesial thickenings of ventral pachycycli) absent or not well developed; seven placids: four dorsal and three ventral; fourteen trichoscalids (7 dorsal and 7 ventral) present, but trichoscalid plates absent; four short, thin, non-articulated outer oral styles and five longer articulated ones, composed of two units.

Neocentrophyes Higgins, 1969—emended diagnosis

Neocentrophyidae with sternal plate on first trunk segment undivided. Lateral terminal spines on segment 11 absent. Females with non-articulated middorsal spinose process on segment 10; males with two pairs of long and flexible penile spines, one on segment 10 and one on segment 11.

Identification key for homalorhagid genera and species of Neocentrophyidae

The description of a new homalorhagid genus as well as the new data provided for *Neocentrophyes* prompted the preparation of an updated identification key for the genera of Homalorhagida. Moreover, also considering the recent description of *Paracentrophyes anurus* and the additional data for *P. quadridentatus* (Zelinka, 1928) (see Sørensen et al. 2010), an updated key to species of *Paracentrophyes* would be useful. Even though *N. intermedius* and *N. satyai* could represent male and female of the same species, both entities are included as two different species in the identification key until a future proper taxonomical revision.

1. Sternal region of first trunk segment completely divided into three plates. Sternal region of terminal trunk segment divided into two plates……..2. (Family: Pycnophyidae).

Sternal region of first trunk segment undivided or partially divided anteriorly. Sternal region of terminal trunk segment formed from a single plate, undivided……3. (Family: Neocentrophyidae).

2. Lateral terminal spines present……Genus: *Pycnophyes.*

Lateral terminal spines absent……Genus: *Kinorhynchus.*

3. Sternal plate on first trunk segment partially divided anteriorly. Lateral terminal spines on segment 11 present. Middorsal spines present on segments 10–11 in both sexes……..5. Genus: *Paracentrophyes.*

Sternal plate on first trunk segment undivided. Lateral terminal spines on segment 11 present or absent. Middorsal spine on segment 10 present in males only. Middorsal spine on segment 11 present in both sexes……4.

4. No lateral terminal spines on segment 11……..7. Genus: *Neocentrophyes.*

Lateral terminal spines on segment 11 present……*Mixtophyes abyssalis* gen. et sp. nov.

5. No setae on segment 10. Short lateral terminal spines (LTS/LT = 7–9 %)……*Paracentrophyes anurus.*

Presence of setae in some positions on segment 10…..6.

6. Paralateral perispinal setae on segment 1. Two pairs of ventrolateral perispinal setae on segment 1……*Paracentrophyes praedictus.*

Paralateral perispinal setae on segment 1 absent. Presence of a single pair of ventrolateral perispinal setae on segment 1 ……*Paracentrophyes quadridentatus.*

7. Flexible and articulated middorsal spines on segments 10 and 11. Long midterminal process and small cuticular bulbous protrusions on segment 11……*Neocentrophyes intermedius.*

Rigid and articulated middorsal spine on segment 11 only, middorsal spinose process on segment 10. Short midterminal process and long cuticular bulbous protrusions on segment 11……*Neocentrophyes satyai.*

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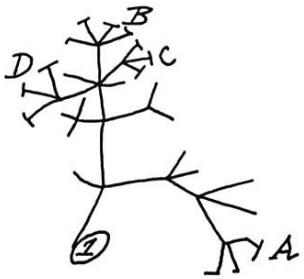
RESULTS

Chapter III

Phylogeny



Morphology disentangles the systematics of a ubiquitous but elusive meiofaunal group (Kinorhyncha: Pycnophyidae)

Nuria Sánchez, Hiroshi Yamasaki, Fernando Pardos, Martin V. Sørensen, Alejandro Martínez

RESUMEN: **La morfología desenmaraña la sistemática de un ubicuo pero esquivo grupo de la meiofauna (Kinorhyncha: Pycnophyidae).** — Los kinorrincos son un grupo de animales bentónicos microscópicos con una distribución mundial presentes en el sedimento marino. El filo está dividido en dos clases, Cyclorhagida y Allomalorhagida, en congruencia con los dos grandes clados obtenidos en análisis filogenéticos recientes. Allomalorhagida aloja más de untercio de las especies descritas, la mayoría de ellas asignadas a la familia Pycnophyidae. En todos los análisis filogenéticos previos sobre el filo se recuperan los dos géneros de Pycnophyidae, *Pycnophyes* y *Kinorhynchus*, como parafiléticos y polifiléticos. El principal problema en estos estudios fue la ausencia de datos moleculares para la mayoría de pycnofidos, debido a la estrecha y muy localizada distribución de muchas especies, habitualmente en el ártico o de aguas profundas. Aquí superamos éste problema añadiendo una partición morfológica con datos de 79 especies de Pycnophyidae, 15 de ellas representadas también por datos moleculares. Los análisis basados en modelos produjeron nueve clados, cada uno de ellos soportado por varias apomorfías morfológicas. En consecuencia, Kinorhynchus fue sinonimizado con Pycnophyes y se describieron ocho nuevos géneros para los restantes clados obtenidos: *Planolimbus* gen. nov., *Cristaphyes* gen. nov., *Higginsia* gen. nov., *Gymnophyes* gen. nov., *Setaphyes* gen. nov., *Fujuriphyes* gen. nov., *Krakenella* gen. nov. and *Godzilliphyes* gen. nov..

INTRODUCTION

Morphology disentangles the systematics of a ubiquitous but elusive meiofaunal group (Kinorhyncha: Pycnophyidae)

Nuria Sánchez, Hiroshi Yamasaki, Fernando Pardos, Martin V. Sørensen, Alejandro Martínez

ABSTRACT Kinorhyncha is a group of benthic, microscopic animals distributed worldwide in marine sediments. The phylum is divided into two classes, Cyclorhagida and Allomalorhagida, congruent with the two major clades recovered in recent phylogenetic analyses. Allomalorhagida accommodates more than one-third of the described species, most of them assigned to the family Pycnophyidae. All previous phylogenetic analyses of the phylum recovered the two genera within Pycnophyidae, *Pycnophyes* and *Kinorhynchus*, as paraphyletic and polyphyletic. A major problem in these studies was the lack of molecular data of most pycnophyids, due to the small and highly localized distribution of many species, often in the Artic and the deep sea. We here overcame the problem by adding a morphological partition with data for 79 Pycnophyidae species, 15 of them also represented by molecular data. Model-based analyses yielded nine clades, that each was supported by several morphological apomorphies. Accordingly, *Kinorhynchus* is synonymized with *Pycnophyes* and eight new genera were described for the remaining recovered clades: *Planolimbus* gen. nov., *Cristaphyes* gen. nov., *Higginsia* gen. nov., *Gymnophyes* gen. nov., *Setaphyes* gen. nov., *Fujuriphyes* gen. nov., *Krakenella* gen. nov. and *Godzilliphyes* gen. nov..

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INTRODUCTION

Kinorhyncha comprises around 200 described species of marine meiobenthic metazoans distributed worldwide (Sørensen, 2013). The phylum belongs to the Ecdysozoa, as part of the Scalidophora (Dunn et al., 2014). All kinorhynchs have a similar external morphology with an elongated body divided into three regions, namely head, neck and trunk. The head is formed by an eversible introvert with appendages, named scalids, arranged in concentric circles around a protrusible mouth cone. The neck consists on a variable number of plates called placids, which vary in number according to the genus and enclose the retracted introvert. The trunk is elongated and consists of eleven segments in adult specimens arranged either as a closed ring or divided into dorsal and ventral plates (Higgins, 1990; Adrianov and Malakhov, 1999; Sørensen and Pardos, 2008).

Based on morphological studies, kinorhynchs were accommodated into 23 genera (Sørensen, 2013; Sánchez et al., 2014a; Sørensen et al., in press) assigned to the orders Homalorhagida (Zelinka, 1896; Chitwood, 1951) and Cyclorhagida (Zelinka, 1896; Higgins, 1964, 1990; Adrianov and Malakhov, 1999). However, during the last decade several specialists in the group joined forces to improve the taxon sampling and increase our knowledge on the kinorhynch phylogeny. These efforts led to a series of molecular phylogenetic analyses based on ribosomal genes (Dal Zotto et al., 2013; Yamasaki et al., 2013). These analyses recovered two large clades in overall consistency with Homalorhagida and Cyclorhagida,

except for the position of the cyclorhagid genus *Dracoderes* Higgins and Shirayama, 1990, which together with the newly described genus *Franciscideres* Dal Zotto et al., 2013 and a yet undescribed genus, grouped with the remaining homalorhagids. This was surprising since these three genera share morphological traits considered as apomorphic for Cyclorhagida (Dal Zotto et al., 2013; Yamasaki et al., 2013). The polyphyly of Cyclorhagida was recently corroborated after a combined analysis of morphological and molecular data, which forced a revision of kinorhynch systematics (Sørensen et al., in press). The phylum is now divided into the classes Cyclorhagida Zelinka, 1896 and Allomalorhagida Sørensen et al., in press, with the latter accommodating the homalorhagid genera *Paracentrophyes* Higgins, 1983, *Neocentrophyes* Higgins, 1969, *Mixtophyes* Sánchez et al., 2014, *Pycnophyes* Zelinka, 1907 and *Kinorhynchus* Sheremetevskij, 1974, together with the genera *Dracoderes* and *Franciscideres* (Sørensen et al., in press). Whereas these analyses resolved the relationship amongst the major clades within Kinorhyncha, they opened up several questions regarding the internal relationships within some of them, especially those corresponding to the two largest kinorhynch families, namely Echinoderidae Bütschli, 1876 and Pycnophyidae Zelinka, 1896.

Pycnophyidae is the second largest family of Kinorhyncha with one-third of the all described species. Pycnophyidae includes the genera *Pycnophyes*, with 56 species, and *Kinorhynchus* with 19 species, making them the second and third most diverse genera of the phylum, respectively (Neuhaus, 2013; Sørensen, 2013; Sánchez et al., 2014b). Pycnophyidae is defined by the presence of one dorsal (tergal) and three ventral (sternal) plates on segment 1, followed by one tergal and two sternal plates on all subsequent segments. *Pycnophyes* differs from *Kinorhynchus* by possessing a pair of large lateral terminal spines on segment 11, which is absent in *Kinorhynchus*. The absence of lateral terminal spines is a very conspicuous character, only shared with the two described species of *Neocentrophyes* (Higgins, 1990; Adrianov and Malakhov, 1999; Sørensen and Pardos, 2008). However, although all phylogenetic analyses recovered Pycnophyidae as a well-supported clade within Allomalorhagida, they failed to recover *Pycnophyes* or *Kinorhynchus* as monophyletic groups, suggesting several losses of the lateral terminal spines within Pycnophyidae (Dal Zotto et al., 2013; Yamasaki et al., 2013; Sørensen et al., in press). Assuming the paraphyly/polyphyly of *Pycnophyes* and *Kinorhynchus*, a new systematic arrangement for the family would be desirable, especially given the high diversity of species within the group and its key phylogenetic position to understand the evolution of Allomalorhagida.

Despite the increased sampling effort by various researchers, the number of sequenced species of Pycnophyidae is still very low. This is not only due to the inherent difficulties in getting good quality sequences from small animals, but mainly due to the low abundances of many species and the restricted distribution areas of many pycnophyids, which are often exclusively known from single collections at the polar regions, deep-sea or even in the stomach content of a shrimp (!) (Adrianov and Malakhov, 1999; Martorelli and Higgins, 2004; Sánchez et al. 2012).

In order to overcome these problems, we here analyze a combined dataset compiling all the available information with potential phylogenetic significance for the family. Our dataset includes three molecular markers for 15 species and 98 morphological characters for all 75 described and 4 undescribed pycnophyids, obtained from the re-examination of all the available type material, literatures and new collections. This approach was adopted in order to further resolve the relationships within the

Pycnophyidae, even though the inclusion of species represented only by morphological partition produced a substantial amount of missing data affecting the nodal supports in certain analyses. However, we consider crucial at this point to provide a new systematic arrangement for the family, which allow a phylogenetic classification criteria of the increasing numbers of newly discovered species in forthcoming surveys.

The goals of this study are as follows: 1) to test the monophyly of *Pycnophyes* and *Kinorhynchus* with molecular and combined data, investigating the effect of alignment on the final topologies; 2) to further explore the relationships of the Pycnophyidae including all described species represented by the morphological partition; 3) search for morphological apomorphies that justify the erection of each of the major recovered clades as new genera.

MATERIAL AND METHODS

Taxon sampling and selection

Kinorhynchs were collected from subtidal sediment samples dredged with a Higgins meiobenthic dredge (Higgins, 1966; Higgins and Thiel, 1988) and extracted alive using the bubbling and blot technique (Higgins and Thiel, 1988; Sørensen and Pardos, 2008) (Table 1). Specimens used in the molecular part of this study were sorted alive and preserved in 100% ethanol. Additional material used for morphological coding was fixed in 4% formalin (see Table 2).

The sequences used in the study were obtained from newly and some previously collected specimens. Hologenophore vouchers were designated (Table 1). Opposite to larger animals (Jörger et al., 2012; Martínez et al., 2013; Di Domenico et al., 2014; Scarpa et al., 2015), DNA extraction in kinorhynchs requires use of the entire animal. However, we

Family	Species	N. Voucher	Locality	Coordinates	Sediment	Depth	18SrRNA	28SrRNA	COI	Literature
Neocentrophyidae	*Pa. anurus*	ZIHU 04295	Amami Island, Japan	21°31,27N 126°57,43E	unknown	339 m	AB738368	AB738369	XXXXX	Yamasaki et al., 2013; this study
	Pa. quadridentatus	not deposited	Naples, Italy	40°47,16N 014°14,91E	Mud	98-99 m	-	LC007076–77	XXXXX	Sørensen et al., in press; this study
Echinoderidae	*E. sensibilis*	not deposited	Shirahama, Japan	33°41,52N 135°20,18E	interstitial-calcareous algae	1 m	LC007047	LC032121	LC032122	Sørensen et al., in press; Yamasaki, in press; this study
	E. rex	not deposited	Seto Inland, Japan	33°54,92N 132°09,12E	Mud	17-25 m	XXXXX	XXXXX	XXXXX	This study
Pycnophyidae	*P. rugosus*	not deposited	Naples, Italy	40°48,98N 014°13,12E	Fine mud	28-30 m	XXXXX	XXXXX	XXXXX	This study
	P. ponticus	not deposited	Naples, Italy	40°48,98N 014°13,12E	Fine mud	28-30 m	XXXXX	XXXXX	XXXXX	This study
	P. flaveolatus	not deposited	Naples, Italy	40°48,98N 014°13,12E	Fine mud	28-30 m	XXXXX	XXXXX	XXXXX	This study
	P. oshoroensis	n.a.	Oshoro, Japan	43°12,42N 149°51,29E	Muddy sand	8 m	AB738372	AB738373	-	Yamasaki et al., 2013
	P. sp. 2012a	ZIHU 04296	Okinawa, Japan	26°40,17N 127°45,26E	Muddy sand	138 m	AB738374	AB738375	-	Yamasaki et al., 2013
	P. sp. 2012b	n.a.	Hokkaido, Japan	44°28,18N 144°04,60E	unknown	204 m	AB738376	AB738377	-	Yamasaki et al., 2013
	P. dentatus	not deposited	Ceuta, Spain	35°53,12N 005° 19,11E	Muddy sand	20 m	LC007052	LC007069	XXXXX	Sørensen et al., in press; this study
	P. robustus	not deposited	Faro, Portugal	37°02,21N 007°47,55W	Zostera	1 m	LC007053	LC007070	XXXXX	Sørensen et al., in press; this study
	P. zelinkaei	not deposited	Naples, Italy	40°46,72N 014°06,79E	Muddy sand	37-53 m	LC007055	LC007071	XXXXX	Sørensen et al., in press; this study
	P. tubulifera	not deposited	Okinawa, Japan	24°22,51N 123°43,44E	Muddy sand	15 m	XXXXX	XXXXX	XXXXX	This study
	P. communis	-	Cesenatico, Italy	44°13,14N 012°28,61E	-	-	KF372867	-	-	Dal Zotto et al., 2013
	P. greenlandicus	-	Disko Island, Greenland	n.a.	-	-	AY428820	-	-	Giribet et al., 2004
	P. kielensis	-	-	n.a.	-	-	U67997	AY863411	-	Petrov and Vladychenskaya, 2005
	K. giganteus	-	Castellammare, Italy	38°02,58N 12°52,97E	-	-	KF372863	-	-	Dal Zotto et al., 2013
	K. yushini	not deposited	Oshoro, Japan	43°12,42N 149°51,29E	Muddy sand	8 m	AB738370	AB73837	LC032123	Yamasaki et al., 2013; Yamasaki, in press; this study

Table 1. Specimen for molecular works and collection data, with GenBank accession numbers. Abbreviations: COI, cytochrome c oxidase subunit I; E, East; m, meters; N, North; ZIHU invertebrate collection of the Hokkaido University Museum.

Table 2. Material examined, including vouchers, type material and additional material. Details of the microscopic techniques and main references used for the coding are included. Abbreviations: *Material collected from the type locality; LM, light microscopy examinations on fixed material; lost, type material does not exist; n.a.loan, type material no available for loan; n.r., type material no required for the study because we are using the hologenophore for the coding; SEM, scanning electron microscopy examinations.

Family	Species	Voucher	Additional	LM	SEM	Type	Literature
Echinoderidae	*E. sensibilis* Adrianov et al., 2002	X	-	X	-	n.r.	-
	E. rex Lundbye et al., 2011	X	-	X	-	n.r.	-
Paracentrophyidae	*Pa. anurus* Sørensen et al., 2010	X	-	X	-	n.r.	-
	Pa. quadridentatus Zelinka, 1928	X	-	X*	-	lost	-
	Pa. praedictus Higgins, 1983	-	-	-	-	X	Higgins, 1983
	M. abyssalis Sánchez et al, 2014	-	-	-	-	X	Sánchez et al., 2014a
	N. intermedius Higgins, 1969	-	-	-	-	X	Higgins, 1969
	N. satyai Higgins, 1969	-	-	-	-	X	Higgins, 1969
Pycnophyidae	*P. rugosus* Zelinka, 1928	X	-	X*	-	lost	-
	P. ponticus Zelinka, 1928	X	-	X*	-	lost	-
	P. flaveolatus Zelinka, 1928	X	-	X*	-	lost	-
	P. oshoroensisis Yamasaki et al., 2012	X	-	X*	-	n.r.	-
	P. sp. 2012a	X	-	X	-	-	-
	P. sp. 2012b	X	-	X	-	-	-
	P. dentatus Reinhard, 1881	X	-	X	-	lost	-
	P. robustus Zelinka, 1928	X	-	X*	-	lost	-
	P. zelinkaei Southern, 1914	X	-	X	-	lost	-
	P. tubuliferus Adrianov, 1989	X	-	X	-	n.r.	-
	P. communis Zelinka, 1908	-	Italy, Spain	X*	X	lost	Zelinka, 1928
	P. greenlandicus Higgins and Kristensen, 1988	-	-	-	-	X	Higgins and Kristensen, 1988
	P. kielensis Zelinka, 1928	-	Germany	-	X*	lost	Zelinka, 1928; Neuhaus, 1993
	K. giganteus Zelinka, 1928	-	Italy	-	X*	lost	Zelinka, 1928
	K. yushini Adrianov, 1989	X	-	X	-	n.r.	-
	P. almansae Sánchez et al., 2014	-	Spain	X*	X*	X	Sánchez et al., 2014c
	P. carinatus Zelinka, 1928	-	Spain	X	X	lost	Zelinka, 1928
	P. chalgap Sánchez et al., 2013	-	South Korea	-	X*	X	Sánchez et al., 2013
	P. cristatus Sánchez et al., 2013	-	South Korea	-	X	X	Sánchez et al., 2013
	P. dolichurus Sánchez et al., 2011	-	Spain	X*	X*	X	Sánchez et al., 2011
	P. farinellii Sánchez et al., 2014	-	-	-	-	X	Sánchez et al., 2014b
	P. frequens Blake, 1930	-	USA	X	X	X	Blake, 1930; Herranz et al., 2014
	P. lageria Sánchez et al., 2014	-	South Korea	X*	X*	X	Sánchez et al., 2014c
	P. norenburgi Herranz et al., 2014	-	USA	X*	X*	X	Herranz et al., 2014
	P. nubilis Sánchez et al., 2014	-	-	-	-	X	Sánchez et al., 2014b
	P. smaug Sánchez et al., 2013	-	South Korea	-	X*	X	Sánchez et al., 2013
	P. sp. nov. 4	-	Japan	X	-	-	-
	P. argentinensis Martorelli and Higgins, 2004	-	-	-	-	X	Martorelli and Higgins, 2004
	P. beaufortensis Higgins, 1964	-	-	-	-	X	Higgins, 1964
	P. ecphantor Higgins, 1983	-	-	-	-	X	Higgins, 1983
	P. borealis Higgins and Korczynski, 1990	-	-	-	-	X	Higgins and Korczinski, 1990
	P. corrugatus Higgins, 1983	-	-	-	-	X	Higgins, 1983
	P. cryopygus Higgins and Kristensen, 1988	-	-	-	-	X	Higgins and Kristensen, 1988
	P. egyptensis Higgins, 1966	-	-	-	-	X	Higgins, 1966
	P. emarginatus Higgins, 1983	-	-	-	-	X	Higgins, 1983
	P. iniorhaptus Higgins, 1983	-	-	-	-	X	Higgins, 1983

P. longicornis Higgins, 1983	-	-	-	-	X	Higgins, 1983
P. neuhausi Martorelli and Higgins, 2004	-	-	-	-	X	Martorelli and Higgins, 2004
P. australensis Lemburg, 2002	-	-	-	-	X	Lemburg, 2003
P. chukchiensis Higgins, 1991	-	-	-	-	X	Higgins, 1991
P. abyssorum Adrianov and Maiorova, 2015	-	-	-	-	n.a.loan	Adrianov and Maiorova, 2015
P. aulacodes Sánchez et al., 2011	-	Spain	X*	X*	X	Sánchez et al., 2011
P. pardosi Sánchez et al., 2013	-	South Korea	-	X*	X	Sánchez et al., 2013
P. sp. nov. 5	-	Japan	X	-	-	-
K. apotomus Higgins, 1983	-	-	-	-	X	Higgins, 1983
K. belizensi Higgins, 1983	-	-	-	-	X	Higgins, 1983
K. deirophorus Higgins, 1983	-	-	-	-	X	Higgins, 1983
K. distentus Higgins, 1983	-	-	-	-	X	Higgins, 1983
K. erismatus Higgins, 1983	-	-	-	-	X	Higgins, 1983
K. fimbriatus Higgins, 1982	-	-	-	-	X	Higgins, 1982
K. langi Higgins, 1964	-	-	-	-	X	Higgins, 1982
K. mainensis Blake, 1930	-	-	-	X*	X	Blake, 1930
K. phyllotropis Brown and Higgins, 1983	-	-	-	-	X	Brown and Higgins, 1983
K. stenopygus Higgins, 1983	-	-	-	-	X	Higgins, 1983
K. trisetosus Higgins, 1983	-	-	-	-	X	Higgins, 1983
P. arctous Adrianov, 1999	-	-	-	-	n.a.loan	Adrianov and Malakhov, 1999
P. barentsi Adrianov, 1999	-	-	-	-	n.a.loan	Adrianov and Malakhov, 1999
P. calmani Southern, 1914	-	-	-	-	lost	Southern, 1914; Zelinka, 1928
P. canadensis Higgins and Korczynski, 1990	-	-	-	-	n.a.loan	Higgins and Korczinski, 1990
P. chilensis Lang, 1953	-	-	-	-	n.a.loan	Lang, 1953
P. faveolus Brown, 1985	-	-	-	-	n.a.loan	Brown, 1985
P. furugelmi Adrianov, 1999	-	-	-	-	n.a.loan	Adrianov and Malakhov, 1999
P. galtsovae Adrianov, 1999	-	-	-	-	n.a.loan	Adrianov and Malakhov, 1999
P. maximus Reimer, 1963	-	-	-	-	lost	Reimer, 1963
P. mokievskii Adrianov, 1995	-	-	-	-	n.a.loan	Adrianov, 1995
P. newguiniensis Adrianov, 1999	-	-	-	-	n.a.loan	Adrianov and Malakhov, 1999
P. newzealandiensis Adrianov, 1999	-	-	-	-	n.a.loan	Adrianov and Malakhov, 1999
P. odhneri Lang, 1949	-	-	-	-	n.a.loan	Lang, 1949
P. parasanjuanensis Adrianov and Higgins, 1996	-	-	-	-	n.a.loan	Adrianov and Higgins, 1996
P. sanjuanensis Higgins, 1961	-	-	-	-	n.a.loan	Higgins, 1961
P. schornikovi Adrianov, 1999	-	-	-	-	n.a.loan	Adrianov and Malakhov, 1999
P. sculptus Lang, 1949	-	-	-	-	n.a.loan	Lang, 1949
P. spitsbergensis Adrianov, 1995	-	-	-	-	n.a.loan	Adrianov, 1995
K. anomalus Lang, 1953	-	-	-	-	n.a.loan	Lang, 1953
K. cataphractus Higgins, 1961	-	-	-	-	n.a.loan	Higgins, 1961; Adrianov and Malakhov, 1999
K. ilyocryptus Higgins, 1961	-	-	-	-	n.a.loan	Higgins, 1961; Boykin, 1965; Adrianov and Malakhov, 1999
K. paraneapolitanus Sheremetevsky, 1974	-	-	-	-	n.a.loan	Sheremetevsky, 1974; Higgins and Adrianov, 1999
K. spinosus Lang, 1949	-	-	-	-	n.a.loan	Lang, 1949
K. rabaulensis Adrianov, 1999	-	-	-	-	n.a.loan	Adrianov and Malakhov, 1999

followed the procedure developed by Yamasaki et al. (2013), which preserves an intact cuticle of the specimens after the DNA extraction, allowing it to be used as a morphological voucher and obtaining molecular and morphological data from the same individual.

The sequences corresponding to the species *Kinorhynchus giganteus* Zelinka, 1928, *Pycnophyes communis* Zelinka, 1908, *Pycnophyes greenlandicus* Higgins and Kristensen, 1988, *Pycnophyes kielensis* Zelinka, 1928, *Pycnophyes oshoroensis* Yamasaki et al., 2012, *Pycnophyes* sp. 2012a and *Pycnophyes* sp. 2012b were downloaded from GenBank. The 18S rRNA sequence available for *Pycnophyes beaufortensis* Higgins, 1964 in GenBank (Accession Number EU669457) was discarded since it was comparatively short and was obtained from specimens collected far away from the known distribution area of the species without designation of vouchers that allow us to confirm its identification (Giribet et al., 2004).

Eight species were designated as outgroups: the cyclorhagids *Echinoderes rex* Lundbye et al., 2010 and *Echinoderes sensibilis* Adrianov et al., 2002 as well as all the described Neocentrophyidae: *Mixtophyes abyssalis* Sánchez et al., 2014, *Neocentrophyes intermedius* Higgins, 1969, *Neocentrophyes satyai* Higgins, 1969, *Paracentrophyes anurus* Sørensen et al., 2010, *Paracentrophyes quadridentatus* Zelinka, 1928 and *Paracentrophyes praedictus* Higgins, 1983. Morphological data were coded for all of them whereas molecular sequences were only available for *E. rex*, *E. sensibilis*, *Paracentrophyes anurus* and *Paracentrophyes quadridentatus*.

DNA extraction and amplification

Total genomic DNA was extracted from each specimens using a DNeasy Tissue Kit (Qiagen, Tokyo), following the protocol of Yamasaki et al. (2013). After DNA extraction, the exoskeleton of each specimen was picked up and used as hologenophore. Nuclear 18S rRNA (18S), 28S rRNA (28S) genes, and mitochondrial cytochrome *c* oxidase subunit I gene (COI) were amplified by PCR using the primer sets listed in Table 3. PCR conditions were same to those in Yamasaki and Fujimoto (2014). All nucleotide sequences were determined by direct sequencing with a BigDye Terminator Kit ver. 3.1 (Life Technologies, Co., USA) and a 3730 DNA Analyzer (Life Technologies, Co., USA). The sequence fragments were assembled by MEGA 5 (Tamura et al., 2011). After assembly, the sequences were deposited in GenBank under accession numbers XXXXXX.

Morphological investigations and morphological matrix

Light microscopy (LM) observations were done on hologenophore vouchers and additional specimens. These were dehydrated through a graded ethanol series, transferred to 100% glycerin and mounted in Fluoromount G®. Whole mounted specimens were examined using an Olympus BX51 light microscope equipped with differential interference contrast optics (DIC).

Scanning electron microscopical (SEM) investigations were performed on dehydrated specimens through a graded ethanol series, transferred to 100% acetone and critical point dried. The dried specimens were then mounted on aluminum stubs, sputter coated with platinum and examined with a JEOL JSM-6335 field emission scanning electron microscope.

Gene	Primer name	Reaction	Primer sequence (in 5'-3' direction)	Direction	Source
18S rRNA	F1	PCR & CS	TACCTGGTTGATCCTGCCAG	Forward	Yamaguchi and Endo (2003)
	R9	PCR & CS	GATCCTTCCGCAGGTTCACCTAC	Reverse	Yamaguchi and Endo (2003)
	F2	CS	CCTGAGAAACGGCTRCCACAT	Forward	Yamaguchi and Endo (2003)
	F3	CS	GYGRTCAGATACCRCCSTAGTT	Forward	Yamaguchi and Endo (2003)
	F4	CS	GGTCTGTGATGCCCTYAGATGT	Forward	Yamaguchi and Endo (2003)
	R6	CS	TYTCTCRKGCTBCCTCTCC	Reverse	Yamaguchi and Endo (2003)
	R7	CS	GYYARAACTAGGGCGGTATCTG	Reverse	Yamaguchi and Endo (2003)
	R8	CS	ACATCTRAGGGCATCACAGACC	Reverse	Yamaguchi and Endo (2003)
28S rRNA	28S-01	PCR & CS	GACTACCCCCTGAATTTAAGCAT	Forward	Kim et al. (2000)
	28Sr	PCR & CS	ACACACTCCTTAGCGGA	Reverse	Luan et al. (2005)
	28Sf	PCR & CS	TGGGACCCGAAAGATGGTG	Forward	Luan et al. (2005)
	28S-3KR	PCR & CS	CCAATCCTTTTCCCGAAGTT	Reverse	Yamasaki et al. (2013)
	28S-2KF	PCR & CS	TTGGAATCCGCTAAGGAGTG	Forward	Yamasaki et al. (2013)
	28jj-3'	PCR & CS	AGTAGGGTAAAACTAACCT	Reverse	Palumbi (1996)
	28S-n05R	CS	CTCACGGTACTTGTTCGCTAT	Reverse	Yamasaki et al. (2013)
	28SR-01	CS	GACTCCTTGGTCCGTGTTTCAAG	Reverse	Kim et al. (2000)
	28S-15R	CS	CGATTAGTCTTTCGCCCCTA	Reverse	Yamasaki et al. (2013)
	28S-3KF	CS	AGGTGAACAGCCTCTAGTCG	Forward	Yamasaki et al. (2013)
	28v-5'	CS	AAGGTAGCCAAATGCCTCATC	Forward	Palumbi (1996)
	28S-42F	CS	GAGTTTGACTGGGGCGGTA	Forward	Yamasaki et al. (2013)
COI	LCO1490	PCR & CS	GGTCAACAAATCATAAAGATATTGG	Forward	Folmer et al. (1994)
	HCO2198	PCR & CS	TAAACTTCAGGGTGACCAAAAAATCA	Reverse	Folmer et al. (1994)
	Ki_COIF	PCR & CS	GGACTGCTTATAGGGTTATTATTCG	Forward	This study
	Ki_COIR	PCR & CS	CCCCCTCCTCTAACCTCATAAAA	Reverse	This study

Table 3. Extraction, primer, PCR setting and sequencing information.

The morphological matrix included 98 characters (75 absence/presence, 23 multistate) coded for 87 taxa (79 pycnophyid species as ingroup and the eight as outgroup) (Table 4). The data matrix was compiled in Mesquite ver. 3.0.1 (Maddison and Maddison, 2007) and uploaded in MorphoBank (Accession number XXXXXX).

The morphological characters were preferably coded after the hologenophore vouchers (13 species) and type material (40 species). Morphological data of *Pycnophyes oshoroensis* and *Pycnophyes* sp. 2012b were obtained from specimens collected together with these used for DNA extraction, because the vouchers were lost (Yamasaki et al., 2013). Vouchers for the GenBank sequences of *Kinorhynchus giganteus*, *P. communis*, *P. greenlandicus* and *P. kielensis* were not designated in the original studies (Aleshin et al., 1998; Giribet et al., 2004; Dal Zotto et al., 2013). The morphology of *P. greenlandicus* was coded after the type material; *K. giganteus*, *P. communis* and *P. kielensis* were coded after newly collected material from their respective type localities since the type material of these species was lost during the Second World War. This was also the case for the type material of the following species, which were coded after the vouchers designated in this study: *Paracentrophyes quadridentatus*, *Pycnophyes dentatus* Reinhard, 1881, *Pycnophyes flaveolatus* Zelinka, 1928, *Pycnophyes ponticus* Zelinka, 1928, *Pycnophyes robustus* Zelinka, 1928, *Pycnophyes rugosus* Zelinka, 1928 and *Pycnophyes zelinkaei* Southern, 1914. The hologenophore of *Pycnophyes* sp. 2012a and the additional specimens of *Pycnophyes* sp. 2012b, *Pycnophyes* sp. nov. 4 and *Pycnophyes* sp. nov. 5 will become type material after the description of the species (Sánchez in prep). The species *Pycnophyes carinatus* Zelinka, 1928 was coded after

newly collected material from the Iberian Peninsula, since its type material was lost. The remaining 25 species included in the study were coded from information on the literature since type material of twenty three species was not available for loan and the type material for *Pycnophyes calmani* Southern, 1914 and *Pycnophyes maximus* Reimer, 1963 do not exist. A detailed list of the material investigated for each species, the microscopic techniques and references used for coding is provided in Table 2.

Morphological characters were coded as bistate or multistate characters. Definitions for each of the characters together with details about the coding and character states are provided in Appendix 1 (several features of the family are shown in Figs. 1-2). Linked characters were coded hierarchically following the principles of "c-coding" (Pleijel, 1995). Each character was coded as "presence/absence" and linked traits were subsequently coded as independent multistate characters. Absent features were coded as "inapplicable" (-) for the subsidiary characters. We discriminated between inapplicable and missing data (coded as question marks in our matrix) in order to facilitate the evaluation of our character coding even though the analysis treated them equally. Continuous characters (total trunk length, maximal sternal width/total trunk length) were coded as categorical by defining six consecutive states and analyzed as unordered (Kitching et al., 1998; Wiens, 2001; Martínez et al., 2014). Several coding methods for morphological characters have been proposed, all of them with problems related to character linkage, hierarchical dependency, missing values and information content (Pleijel, 1995; Wilkinson, 1995; Kitching et al., 1998; Lipscomb et al., 1998). The problems associated with the "c-coding" are the effect of "inapplicable" characters on the tree topology and the hierarchical coding that might inflate the nodal support for certain clades. Given that the problem remains open and "c-coding" has been widely chosen in previous analyses in other meiofaunal groups (Worsaae, 2005; Di Domenico et al., 2014; Martínez et al., 2014), this method is chosen for the present study.

Several additional characters were considered during some of our preliminary analyses, but they were finally excluded from the final matrix because they were only available for few species. These characters included the presence of different juveniles stages, the distribution of the introvert trichoscalids by sectors, number of dorsal sensory spots by segment, the presence of cuticular tufts surrounding the penile spines, the presence of ornamentation on the lateral terminal spines, the number of canals at the base of each of the lateral terminal spines, the presence of cuticular ornamental wrinkles and presence and number of cuticular ridges.

Other characters considered in previous taxonomic studies on Pycnophyidae (e.g. by Zelinka, 1928; Higgins, 1983; Sørensen et al., 2012; Yamasaki et al., 2012; Sánchez et al., 2013; Sánchez et al., 2014a, 2014b, 2014c) were discarded due to their subjectivity or because they exhibited a continuous range of variation. Some of these character are, e.g., shape of the anterior dorsal margin (varying continuously between the proposed strongly denticulate and serrated character states), the anterior ventral segment ornamentation of midsternal plate (difficult to discriminate between straight, rounded or heart-shaped), ornamentation in the anterior dorsal area of segment 1 (varying between smooth and reticulated), cuticular surface along the entire trunk (either covered by scattered hairs or scale-like hairs, indistinguishable under LM), presence of intracuticular pores along the surface (character only visible with light microscopy and rarely mentioned in the literature), shape of the posterior ventral margin of segment 10 (character states pointed/rounded vary continuously). Finally, a few characters were removed because they exhibit

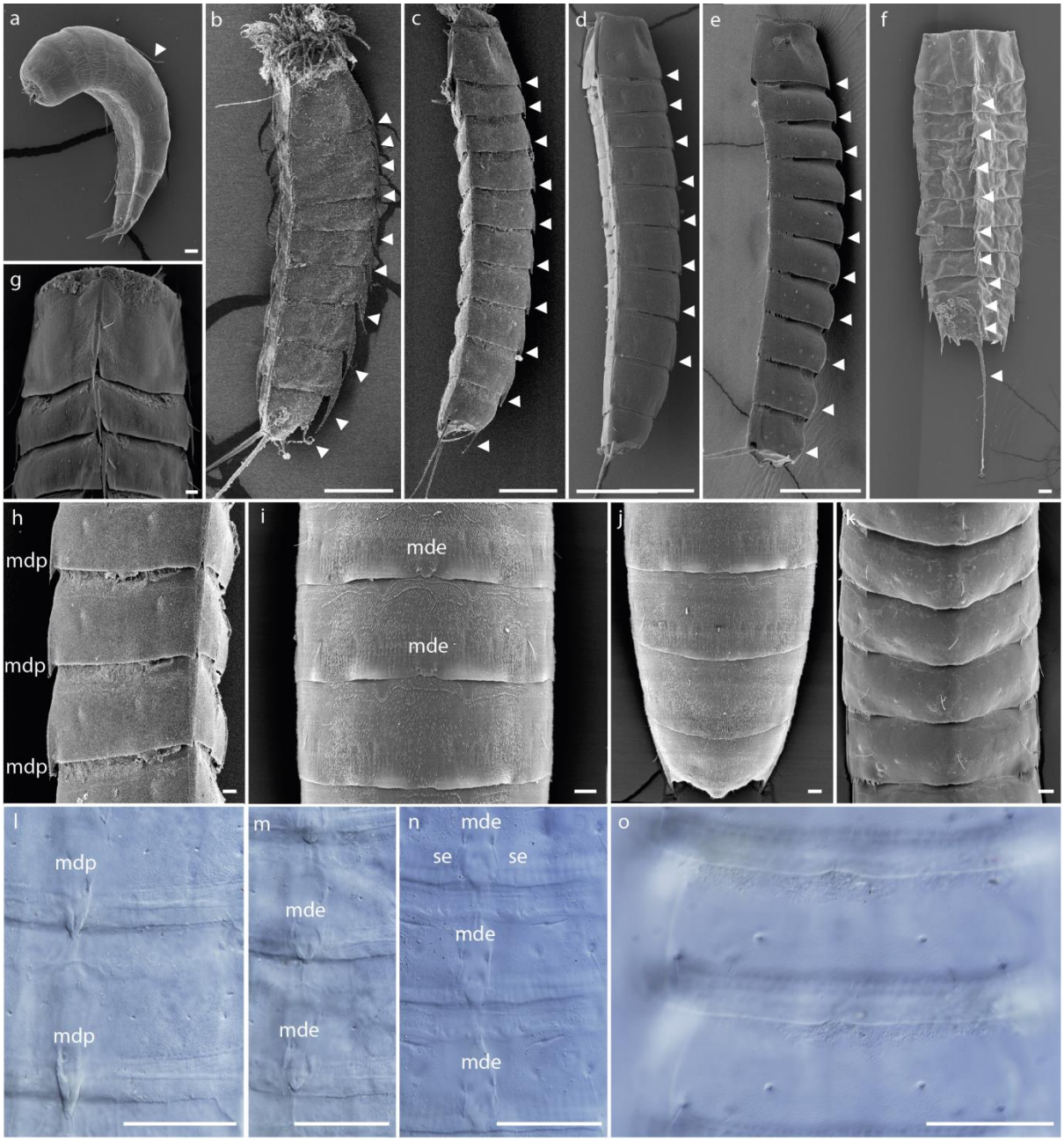

Fig 1. SEM and LM photographs on morphology of Kinorhyncha, focused on the middorsal structure specializations and their character states. (a) *Echinoderes* (outgroup genus), lateral overview, showing middorsal spines (scale bar: 10 μm). (b) *Mixtophyes abyssalis* (outgroup species), lateral overview, showing middorsal spinose processes (scale bar: 100 μm). (c) *Pycnophyes cristatus*, lateral overview, showing keel-like middorsal processes (scale bar: 100 μm). (d) *Pycnophyes frequens*, lateral overview, showing middorsal elevations (scale bar: 100 μm). (e) *Pycnophyes pardosi*, lateral overview, showing smooth middorsal margins (scale bar: 100 μm). (f) Juvenile stage of Pycnophyidae, dorsal overview, showing middorsal processes increasing in length towards the posterior segments (scale bar: 10 μm). (g) *Paracentrophyes quadridentatus* (outgroup species), dorsal view of segments 1-3, detail of middorsal spinose processes (scale bar: 10 μm). (h) *Pycnophyes cristatus*, lateral view of segments 3-5, detail of keel-like middorsal processes surpassing the posterior margin of the segments (scale bar: 10 μm). (i) *Kinorhynchus mainensis*, dorsal view of segments 5-7, detail of middorsal elevations that no surpass the posterior margin of segments 5-6 and smooth middorsal margin of segment 7 (scale bar: 10 μm). (j) *Kinorhynchus mainensis*, dorsal view of segments 7-10, detail of smooth middorsal margins (scale bar: 10 μm). (k) *Pycnophyes pardosi*, dorsal view of segments 3-7, detail of smooth middorsal margins flanked by paradorsal setae (scale bar: 10). (l) *Pycnophyes dentatus*, dorsal view of segments 7-8, detail of middorsal processes surpassing the posterior margin of the segments (scale bar: 50 μm). (m) *Pycnophyes dentatus*, dorsal view of segments 5-6, detail of middorsal elevations that no surpass the posterior margin of the segments (scale bar: 50 μm). (n) *Pycnophyes almansae*, dorsal view of segments 6-8, detail of middorsal elevations that no surpass the posterior margin of the segments flanked by pairs of paradorsal setae (scale bar: 50 μm). (o) *Pycnophyes lageria*, dorsal view of segments 7-8, detail of smooth middorsal margins (scale bar: 50 μm). Abbreviations: mde, middorsal elevation; mdp, middorsal process; se, seta; head arrows mark the middorsal structure specialization on each segment.

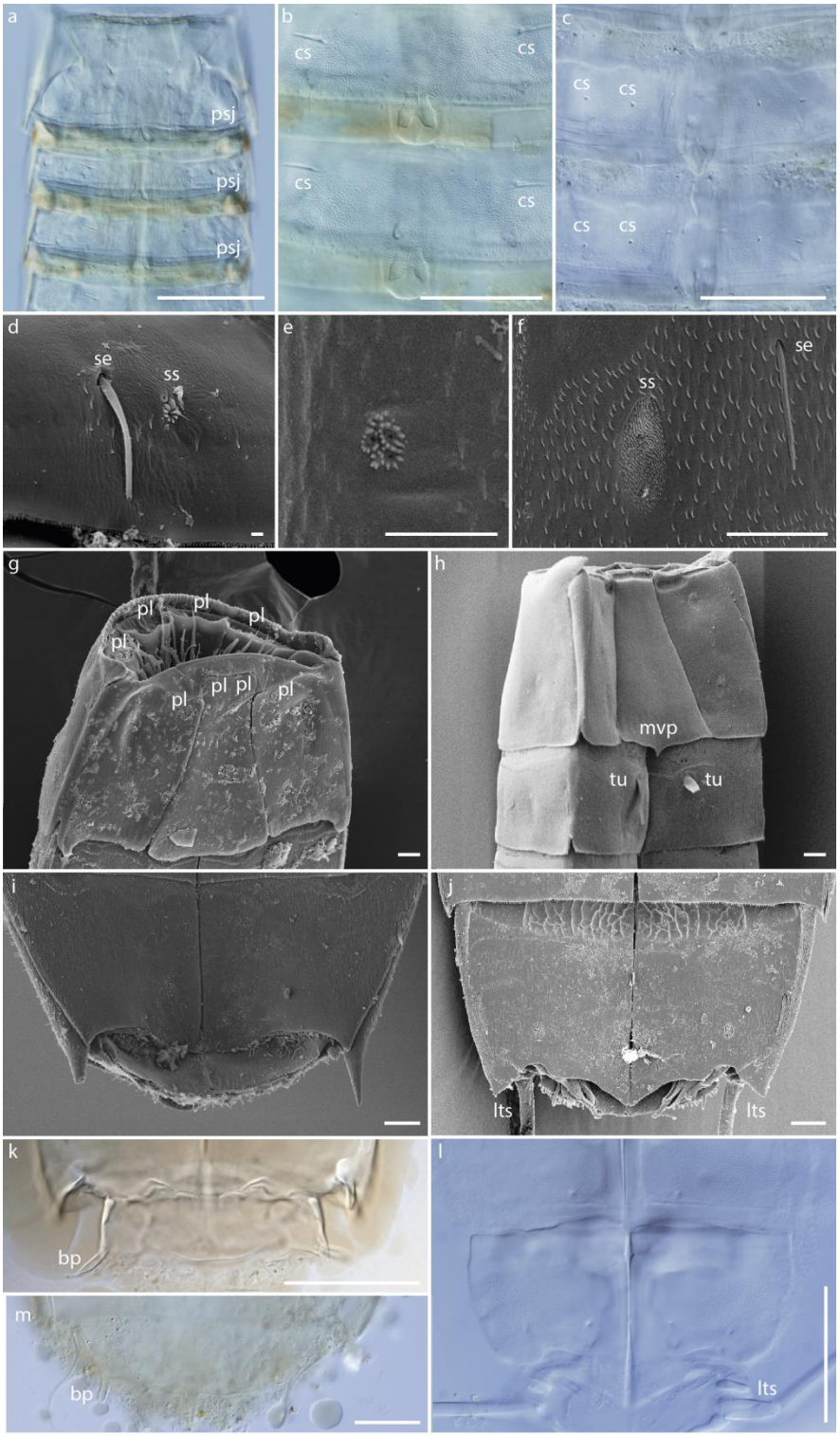

Fig 2. SEM and LM photographs on morphology of Kinorhyncha, focused on kind of muscular scars, kind of sensory spots, placids and ventral view of segment 1, lateral terminal spines and protuberances. (a) *Pycnophyes aulacodes*, dorsal view of segments 1-4, detail of ball and socket joints in the articulation site of tergal and sternal plates (scale bar: 100 µm). (b) *Pycnophyes aulacodes*, dorsal view of segments 5-7, detail of groove-shaped cuticular scars (scale bar: 50 µm). (c) *Pycnophyes dentatus*, dorsal view of segments 3-5, detail of scattered dot-shaped cuticular scars (scale bar: 50 µm). (d) *Pycnophyes lageria*, ventral view of segment 8, detail of *Nanaloricus*-like sensory spot and setae (scale bar: 1 µm). (e) *Paracentrophyes anurus*, ventral view of segment 1, detail of three sensory spots arranged together (scale bar: 10 µm). (f) *Pycnophyes norenburgi*, ventral view of segment 7, detail of typical sensory spot with two pores surrounded by a high number of papillae (scale bar: 10 µm). (g) *Pycnophyes carinatus*, ventral view of segment 1, with one midsternal and two episternal plates, detail of ventral placids and even posterior margin of midsternal plate (scale bar: 10 µm). (h) *Pycnophyes frequens*, ventral view of segments 1-2, detail of the midventral projection on the posterior margin of midsternal plate and the typical ventral tubes of males on segment 2 (scale bar: 10 µm). (h) *Kinorhynchus mainensis*, ventral view of segments 10-11, without lateral terminal spines (scale bar: 10 µm). (i) *Pycnophyes*

tubuliferus, ventral view of segments 10-11, with lateral terminal spines (scale bar: 10 µm). (k) *Kinorhynchus belizensis*, ventral view of segments 10-11, detail of protuberances in the same position as the lateral terminal spines should be located (scale bar: 20 µm). (l) *Pycnophyes ponticus*, ventral view of segments 10-11, detail of lateral terminal spines and their insertions and base (scale bar: 50 µm). (m) *Neocentrophyes intermedius*, ventral view of segments 10-11, detail of protuberances in the same position as the lateral terminal spines should be located (scale bar: 20 µm). Abbreviations: bp, bulbous protuberance; bsj, ball and socket joints; cs, cuticular scar; lts, lateral terminal spines; mvp, midventral process; pl, placid; se, seta; ss, sensory spot; tu, tube.

sexual dimorphism, including the presence/absence of ventrolateral setae on segment 2; and the presence/absence of lateroventral setae on segments 6 and 8, which are present in all species of the family and therefore not informative in our analyses.

Phylogenetic analyses

Morphological data were available for a total of 87 species, including 79 Pycnophyidae and eight outgroups; whereas molecular data were obtained for 19 taxa (15 ingroups, 4 outgroups). Molecular and morphological partitions were analyzed independently and as two different combined datasets: a restricted dataset, which included only the species represented by both molecular and morphological partitions (19 species); and a total dataset, which gathered all the available information for the 87 taxa included in this study.

The morphological partition was analyzed independently under static homology by parsimony, whereas the molecular, combined and total datasets were analyzed under model-based methods (Maximum likelihood and Bayesian), as well as dynamic homology and parsimony (except for the total dataset). Details of each of the analyses are provided below.

Maximum parsimony analyses

The morphological data was analyzed under parsimony using the software PAUP ver. 4.0b10 (Swofford, 2003). The 98 characters were equally weighed and treated as unordered. Heuristic searches started with 100 Wagner trees and continued with timed searches using branch-swapping algorithm of tree bisection reconnection (TBR). Nodal support was estimated via Jackknife analysis with 37% of deletions for a +50% majority-rule consensus tree. Alternative analyses treating the character 15, 16 and 17 (composition of segments 1, 2 and 11) as ordered were performed, yielding very similar topologies and support values.

A dynamic homology approach was applied to the molecular and combined datasets using the software POY ver. 4.1.2. (Wheeler et al., 2006; Varón et al., 2010). The sequences of the ribosomal genes 18S rRNA and 28S rRNA were divided into 29 and 50 homologous regions respectively, after their secondary structure (Clark et al., 1984; Hendriks et al., 1988) and incorporated to the analysis via direct optimization. Since the COI sequences showed no variation in length, they were treated as prealigned.

The searches were run under six analytical parameter sets named 111, 121, 211, 221, 3211 and 3221 (Boyer et al., 2007; Giribet et al., 2012) in order to test the sensitivity of our results to different aligning patterns (Wheeler, 1995). To ensure the recovery of all the optimal trees for each parameter set, we run timed searches (combining multiple Wagner trees, followed by subtree pruning and regrafting (SPR) + tree bisection and reconnection (TBR) + ratchet and tree fusing) followed by additional rounds of sensitivity analysis tree fusing (SATF) (Giribet, 2007). The results for each round of tree fusing were compared to check for the stability in the results. Searches were terminated when the same result was found multiple times for a parameter set. The congruence among different parameter sets was

shown on the most parsimonious tree using Navajo rugs (Giribet, 2003).

In order to identify the optimal analytical parameter set, we compared the incongruent length differences index (wILD) (Wheeler, 1995; Sharma et al., 2011) for each set of parameters. The index was calculated by subtracting the steps obtained from the analyses of each individual molecular and morphological partition to the steps obtained from the analyses of the combined dataset. Optimal trees for each partition were evaluated with identical search strategies to those described above. The resulting wILD values are shown in Table 5. Nodal support of the favored tree was assessed via jackknife (Felsenstein, 1985), calculated with 1000 replicates and a default 0.37 substitution rate. Since resampling techniques are meaningless under dynamic homology, only those characters that were static a priori (morphology and COI) and the dynamic characters with no indels (evaluated by using the command auto_sequence_partition in POY) were resampled during jackknife calculations (Wheeler et al., 2006).

Model-based analyses

Molecular, combined and total datasets were analyzed under maximum likelihood and Bayesian approaches. The 18S rRNA and 28S rRNA gene fragments were aligned independently using the L-ins-I algorithm implemented in the software MAFFT ver. 7.058 (Katoh et al., 2002; Katoh et al., 2010), resulting in 1806 aligned positions for the 18SrRNA and 3410 positions for the 28SrRNA. Gaps were treated in two different ways in order to produce two different analyses. In one analyses, gaps were removed using the software TrimAl (Capella-Gutiérrez et al., 2009), leaving 1149 ungapped positions for the 18SrRNA and 2678 positions for the 28SrRNA. In an alternative analysis, only ambiguously aligned positions were culled from the alignments using Gblocks ver. 0.91b (Castresana, 2000) and allowing smaller final blocks, gap position within the final blocks and less-strict flanking positions. The initial alignments were reduced to 1765 position in the 18SrRNA and 3184 positions in the 28SrRNA after this treatment. The alignment of COI protein-encoding sequences was trivial since it exhibited no variation in length. However, the sequences were aligned using MAFFT ver. 7.058 and checked for gaps and reading frame before using them in further analyses. Each molecular partitions was analyzed independently using Bayesian Inference (BI) and Maximum Likelihood (ML) before they were combined using Sequence Matrix software (Vaidya et al., 2011) and analyzed partitioned. Results obtained from the analyses of each individual gene partition were congruent with the combined analyses, independently to the analyses method and the alignment strategies. The topologies obtained from 18S rRNA and 28S rRNA analyses were nearly identical, whereas those obtained with the COI were very similar as well, except for the lack of resolution in the most basal splits, which resulted in polytomies.

Maximum Likelihood trees were calculated using RAxML ver. 8.1.11 (Stamatakis et al., 2008). Each molecular partition was analyzed under a general time reversible model with corrections for a discrete gamma distribution (GTR+Γ), whereas the Mkv model was selected for the morphological partition (Lewis, 2001). Nodal supports were calculated by non-parametric bootstrap with 1000 replicates and a GTR+Γ model (Felsenstein, 1985).

Bayesian analyses were computed with MrBayes ver. 3.2.2 (Ronquist and Huelsenbeck, 2003). A GTR+Γ model was selected for each gene after the Akaike information criterion (AIC) estimated with jModeltest (Posada, 2008). The morphological partition was analyzed with a Mk1 model (Lewis, 2001). Two independent runs with four Markov chains (three heated and one cold) were submitted in each analysis. The number of generations was set to

50.000.000, and each chain was sampled every 1000 generations. The first 10.000.000 generations were discarded as burn-in. Consensus trees were built after the convergence of the chains was assessed using Tracer ver. 1.4.1 (Rambaut and Drummond, 2007).

All alignments and phylogenetic analyses in this study were run using the Cipres Phylogenetic Portal (Miller et al., 2010).

18s	28s	COI	Mol	Combined	wILD	
3221	1645	6235	1658	9588	9880	0,00779352
3211	1289	4989	1239	7559	7853	0,00904113
221	1413	5634	1239	8330	8624	0,00846475
211	883	3456	829	5193	5485	0,00948040
121	1160	4277	1239	6719	7011	0,00998431
111	753	2746	829	4353	4642	0,01055579

Table 5. Tree length for different data partitions analyzed and the incongruent length differences (wILD) between datasets. Abbreviations: COI, cytochrome c oxidase subunit I; 18s, 18S rRNA; 28s, 28S rRNA; Mol, all molecular partitions; Morph, morphological partition.

Ancestral character state reconstruction

Ancestral character states were reconstructed with the software Mesquite ver. 3.0.1 (Maddison and Maddison, 2007), using parsimony as optimal criteria. The most relevant characters were traced on the trees (see Results and Discussion).

RESULTS

1. Molecular and combined analyses: *Kinorhynchus* and *Pycnophyes* non monophyletic.

The topologies and nodal support values yielded by parsimony and model-based methods were highly congruent among the molecular and combined analyses. The character congruence analysis favored the parameter set 3221 (wILD index=0.007794) for the dynamic homology analyses, although the topologies yielded by all sets of parameters were nearly identical (Fig. 3a). The monophyly of Pycnophyidae received a maximum nodal support by dynamic homology and model-based methods, with *Kinorhynchus giganteus* and *K. yushini* Adrianov, 1989, always recovered as nested amongst species of *Pycnophyes*, independent to the approach. The overall topology of these trees yielded two major clades with *K. yushini* either recovered as sister of the remaining Pycnophyidae (with maximum nodal support under parsimony) or forming a polytomy next to them (under model-based).

Within the first major clade (Parsimony Jackknife, PJN: 0.92; Bayesian posterior probability, BPP: 1; Maximum likelihood bootstrap, ML: 100) (Fig. 3a-b), a subclade with *Pycnophyes oshoroensis* and *Pycnophyes tubuliferus* Adrianov, 1989 branched off with maximum nodal support in all the analyses, and as sisters to a clade including *P. ponticus*, *P. rugosus*, *P. robustus* and *Pycnophyes* sp. 2012a (PJN: 0.79; BPP: 1; ML: 83).

The second clade, formed by *Kinorhynchus giganteus*, *Pycnophyes communis*, *P. dentatus*, *P. flaveolatus*, *P. greenlandicus*, *P. kielensis*, *P. zelinkaei* and *Pycnophyes* sp. 2012b was recovered with maximum nodal support values under BI analyses and relatively lower support under ML analyses and parsimony (PJN: 0.66; BPP: 1; MLB: 75) (Fig. 3a-b). This clade always split off into two subclades. In one of them, *K. giganteus* was consistently recovered next to *P. communis* with high nodal support (PJN: 0.95; BPP: 1; MLB: 90), both nested in a monophylum including *P. greenlandicus* as the sister taxon of *K. giganteus* and *P. communis* (PJN: 0.98; BPP: 0.82; MLB: 76), and *P. zelinkaei* (PJN: 0.89; BPP: 1; MLB: 96) and *Pycnophyes* sp. 2012b branching off successively next to them (PJN: 0.93; BPP: 1; MLB: 100). Under the molecular analyses, the positions of *P. greenlandicus* and *P. zelinkaei* were shifted. The second subclade included *P. dentatus*, *P. flaveolatus* and *P. kielensis* with the highest nodal supports for the clade and its internal relationships in all the analyses.

Fig 3. Phylogenetic tree of combined and molecular data. (a) Phylogenetic tree based on parsimony and direct optimization analyses of the combined dataset with POY under parameter set 3221. Black color in Navajo rugs indicate monophyly of a given node under each parameter set. Numbers inside the Navajo rugs indicate jacknife support for each parameter set. (b) Phylogenetic tree based on model-based methods. Tree topology based on the combined Bayesian analyses under less stringent cleaning. All tree topologies from molecular (gray color) and combined (black color) dataset highly congruent. Only nodal support above BPP = 0.5 or MLB = 50 are displayed. Asterisks indicate maximum nodal support (BPP = 1.00, MLB= 100). Nodal supports located in the top refer to the results yielded by the sequences with less stringent cleaning. Nodal supports located at the bottom refer to the results yielded by the sequences with high stringent cleaning.

2. Inclusion of all described pycnophyids: morphology to the rescue of the molecular partitions.

All the model-based analyses on the total dataset yielded at least seven clades independently to the analyses method and the alignment strategies, with the few exceptions mentioned below (Fig. 5). This overall arrangement was congruent amongst the molecular and combined analyses (Fig. 3), with lower nodal support in the ML analyses, more affected by the high amount of missing data. The morphological dataset yielded a similar topology as well (Fig. 4). The seven clades were named as follows: "*Pycnophyes*", "*Gymnophyes*", "*Planolimbus*", "*Higginsia*", "*Cristaphyes*", "*Fujuriphyes*" and "*Setaphyes*".

Under Bayesian analyses of the combined dataset, "*Pycnophyes*" clade (BPP: 0.85), "*Gymnophyes*" clade (BPP: 0.93) and the species *Pycnophyes zelinkaei*, *Kinorhynchus ilyocryptus* Higgins, 1961 and *Pycnophyes* sp. 2012b formed a clade (BPP: 0.54). Clades of "*Pycnophyes*" + "*Gymnophyes*" and "*Pycnophyes*" + "*Gymnophyes*" + *Kinorhynchus ilyocryptus* were recovered with BPP 0.57 and BPP 0.52, respectively. The latter clade together with *P. zelinkaei*, as well as the latter clade, *P. zelinkaei* and *Pycnophyes* sp. 2012b showed close relationships with BPP: 0.84 and BPP: 0.54, respectively. The clades "*Planolimbus*" (BPP: 0.58), "*Higginsia*" (BPP: 0.56) and "*Cristaphyes*" (BPP: 0.77), formed another monophylum (BPP: 0.73). The latter two clades showed a closer relationship with BPP: 0.59. The other two clades, named "*Fujuriphyes*" and "*Setaphyes*", were recovered with BPP >0.99. The remaining pycnophyids were recovered as a polytomy near the root of the tree.

As the internal relationships of "*Planolimbus*", "*Higginsia*", "*Fujuriphyes*" and "*Setaphyes*" were well resolved. The clade "*Planolimbus*" split into two subclades, named "*P. chalgap-P. faveolus"* (BPP: 0.89; with three species forming a polytomy) and "*P. emarginatus-K. fimbriatus"* (BPP: 0.51; excluding *P. emarginatus* Higgins, 1983 BPP: >0.99). Contrarily, the relationships within the clades "*Pycnophyes*", "*Gymnophyes*" and "*Cristaphyes*" resulted in polytomies with few subclades consistently recovered among analyses. We recovered two subclades within "*Pycnophyes*": "*P. communis-K. paraneapolitanus*" (BPP: 0.95); and "*P. frequens-P. schornikovi"* (BPP:

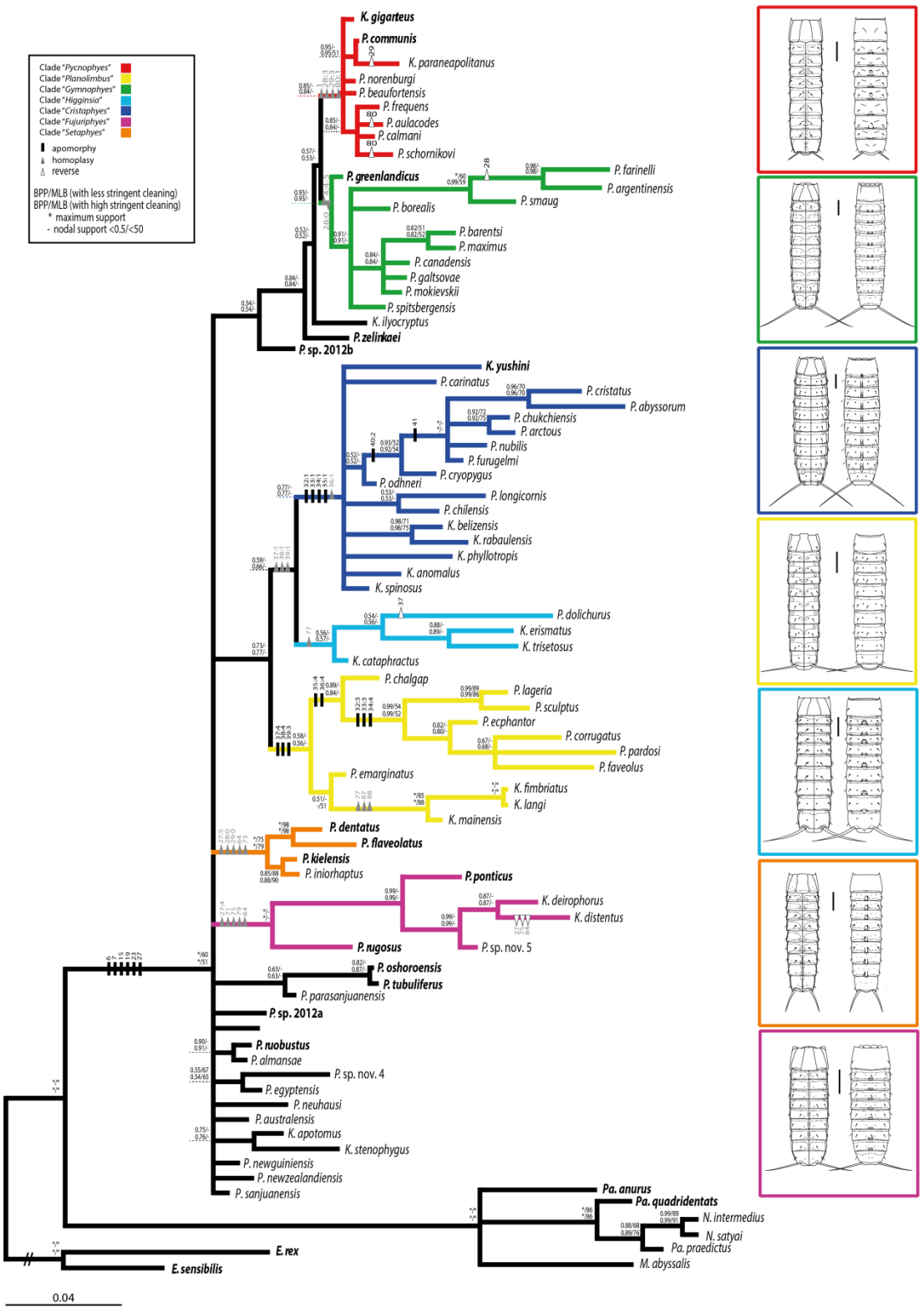

Fig 5. Phylogenetic relationships of Pycnophyidae from the total dataset. Tree topology based on the total Bayesian analyses. Tree topologies from total ML analyses were highly congruent. Only nodal support above BPP = 0.5 or MLB = 50 are displayed. Asterisks indicate maximum nodal support (BPP = 1.00, MLB= 100). Nodal supports located in the top refer to the results yielded by the sequences with less stringent cleaning. Nodal supports located at the bottom refer to the results yielded by the sequences with high stringent cleaning. Black bars correspond to apomorphies. Gray triangles correspond to homoplastic characters used for characterize the clade. White triangles correspond to reversions. Each bar or triangle correspond to the character specified by the numbers outside the bar. Numbers after characters correspond to character state for multistate characters. Names in bold mark the species coded with molecular and morphological data. Drawings correspond to Pycnophyidae whose morphology fit with the diagnosis of each genera (scale bars: 100 µm).

0.85). "*Gymnophyes*" appeared as a polytomy with two subclades, "*P. smaug-P. farinellii*" (BPP: >0.99) and "*P. barentsi-P. mokievskii*" (BPP: 0.84), branching off next to *P. borealis* and *P. spitsbergensis*, and *P. greenlandicus* diverging next to all of them. Within the "*Cristaphyes*" two well-supported subclades were yielded in the polytomy: "*K. belizensis-K. rabaulensis*" (BPP: 0.98) and "*P. cristatus-P. cryopygus"* (BPP: 0.93).

The topologies under maximum likelihood were similar with only a few differences. *Pycnophyes australensis* was recovered within the clade "*Setaphyes*"; the two major subclades in "*Planolimbus*" were paraphyletic; and *Pycnophyes parasanjuanensis* Adrianov and Higgins, 1996, *Pycnophyes sanjuanensis* Higgins, 1961 and *Pycnophyes* sp. 2012b branched off next to the large clade formed by "*Pycnophyes*", "*Setaphyes*", *P. zelinkaei* and *Kinorhynchus ilyocryptus*. One additional clade was recovered, accommodating *Kinorhynchus apotomus* Higgins, 1983, *Pycnophyes almansae* Sánchez et al., 2014, *Pycnophyes egyptensis* Higgins, 1966, *Pycnophyes neuhausi* Martorelli and Higgins, 2004, *Pycnophyes newguiniensis* Adrianov, 1999, *Pycnophyes newzealandiensis* Adrianov, 1999, *P. robustus*, *Pycnophyes* sp. 2012a and *Pycnophyes* sp. nov. 4 (all forming a polytomy under BI, branching next to the root of Pycnophyidae).

Parsimony analyses with PAUP on the morphological dataset yielded a single most parsimonious tree with 642 steps (Fig. 4). Bootstrap values are lower at the most inclusive clades, increasing towards the derived splits of the tree. The topology obtained was mostly congruent with those recovered in the other analyses. The parsimony analyses failed recovering "*Gymnophyes*", with several species splitting off serially near the root of the Pycnophyidae. The clade "*Higginsia*" was polyphyletic, with *Kinorhynchus cataphractus* Higgins, 1961, *Kinorhynchus erismatus* Higgins, 1983 and *Kinorhynchus trisetosus* Higgins, 1983 recovered together with "*Cristaphyes*". "*Setaphyes*" was monophyletic, but nested within a clade including the remaining Pycnophyidae, except for *K. cataphractus*, *K. ilyocryptus*, *Kinorhynchus stenopygus* Higgins, 1983, *P. zelinkaei* and *Pycnophyes* sp. 2012a.

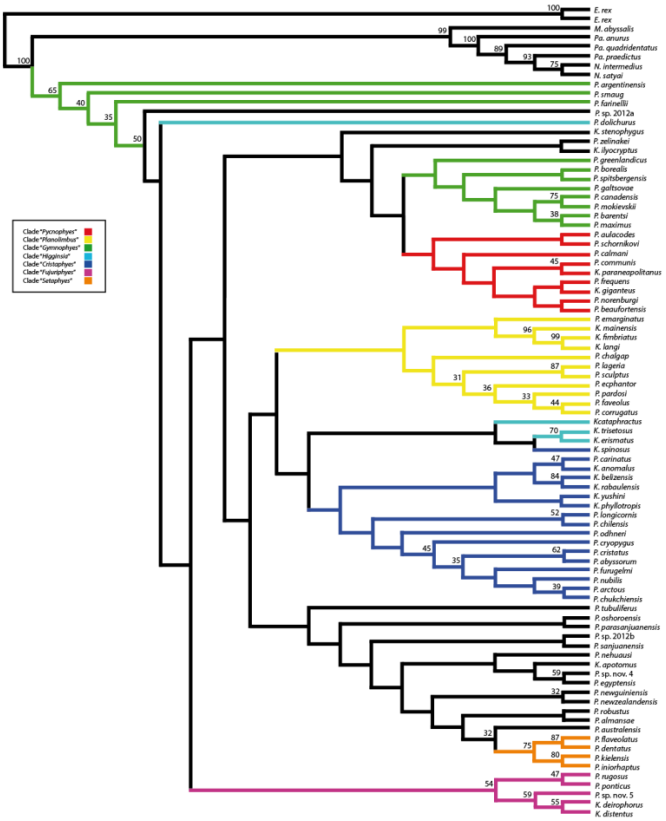

Fig 4. Phylogenetic tree based on maximum parsimony with PAUP of morphological dataset. Nodal support was estimated via Jackknife analysis. Only nodal support above 30 are displayed.

3. Character transformations.

Pycnophyidae was supported by the transformation of six characters (see Fig. 5). Even though the internal topologies of Pycnophyidae yielded by the different analyses were not exactly the same, both were congruent and showed congruent species compositions (see also *Taxonomic implications of the analysis: new classification of Pycnophyidae*).

The clade "*Planolimbus*" was supported by the presence of smooth posterior dorsal margin on

segments 7, 8 and 9 (unique apomorphies, characters 37, 38 and 39). The clade split off into two subclades, each of them supported by unique apomorphies. The subclade rooted by *Pycnophyes chalgap* Sánchez et al., 2013 is supported by the presence of smooth posterior dorsal margin on segments 5 and 6 (characters 35 and 36). The smooth posterior dorsal margins were also present on segments 2, 3 and 4 (unique apomorphies, characters 32, 33 and 34) in all the species of this subclade, except for *P. chalgap*, which instead had middorsal elevations. Within "*Planolimbus*", the presence of middorsal elevation (plesiomorphic condition of Pycnophyidae) was retained on segments 2, 3 and 4 in *P. chalgap* and the subclade rooted by *Pycnophyes emarginatus*. The subclade rooted by *Kinorhynchus mainensis* Blake, 1930 was supported by the presence of ventrolateral setae, absence of ventromedial setae and three pairs of ventral sensory spots on segment 9 (characters 77, 87, 88), although all these characters show low homoplasy.

As for "*Cristaphyes*", it was supported by the presence of middorsal processes on segments 2, 3, 4 and 5 (characters 32, 33, 34 and 35, unique apomorphies). The subclade rooted by *Pycnophyes cryopygus* Higgins and Kristensen, 1988 was supported by the presence of middorsal process on segment 10 (character 40, unique apomorphy). The subclade "*P. cristatus-P. furugelmi*" was supported by the presence of a conspicuous middorsal process on segment 10 extending beyond terminal end of the trunk (character 41, unique apomorphy). Additionally, "*Cristaphyes*" was supported by several apomorphies, that however appeared homoplasious, as the presence of middorsal processes on segment 6 (character 36; also present in *Kinorhynchus trisetosus*), and on segments 7, 8 and 9, present in all the species of "*Higginsia*" as well as in *P. dentatus* and *P. flaveolatus* belonging to the clade "*Setaphyes*" (two gains in total) (characters 37, 38 and 39; except on segment 7 in *Pycnophyes dolichurus* Sánchez et al., 2011).

"*Higginsia*" was defined by a combination of character traits: middorsal elevations never surpassing the posterior margin of the segment on segments 2-5, middorsal processes surpassing the margin of some segments 6-9, and presence ventrolateral setae on segment 5 (characters 32-39, 73). Middorsal processes on segments 7, 8 and 9 (characters 37, 38 and 39; except on segment 7 in *Pycnophyes dolichurus*, which presents a middorsal elevation) were present in all species of "*Cristaphyes*", as well as in *P. dentatus* and *P. flaveolatus* (belonging to "*Setaphyes*"). Only a single apomorphy, i.e. the presence of ventrolateral setae on segment 9 (character 77) supported the clade, but this character evolved convergently three other times outside "*Higginsia*".

Several apomorphies were found for the clade "*Gymnophyes*", although all of them showed a certain degree of homoplasy in other clades. "*Gymnophyes*" was supported by a combination of characters: absence of paradorsal setae along the whole trunk segments (if present it occurred on a single segment only) (characters 42-51) together with the presence of middorsal elevations on all segments 2-9 (characters 32-39). Other Pycnophyidae lack paradorsal setae in certain segments but the absence of paradorsal setae on all segments was only shared by the species recovered into "*Gymnophyes*", and in the subclades "*P. cristatus-P. furugelmi*" and "*P. sculptus-P. lageria*". Additional apomorphic characters were the presence of scattered dot-shaped dorsal cuticular scars along the trunk (character 28; with a single reversion in "*P. farinellii-P. argentinensis*"; and a gain in "*Setaphyes*"); and a total trunk length over 800 µm (character 4, recovered in additional clades by six gains).

"*Setaphyes*" was supported by a combination of apomorphies, with homoplasy in other clades:

presence of paradorsal setae on segments 2-9 (characters 43-50) and presence of lateroventral setae on segments 2-10 (character 61-67). Moreover, the apomorphic presence of lateroventral setae and absence of ventrolateral setae on segment 5 was shared by all species within "*Setaphyes*" and *Pycnophyes lageria* Sánchez et al., 2014 (characters 64, 73). The presence of pachycycli with well-developed ball and socket joints on segments 2-3 only was shared with three other species (character 27; two additional losses). The presence of dorsal and ventral scattered dot-shaped cuticular scars was also recovered in "*Gymnophyes*" (characters 28, 29, one and two additional gains). *Pycnophyes australensis* Lemburg, 2002 was recovered into this clade in the ML analyses, and present many of the characters mentioned above, and only differed in the distribution of pachycycli with ball and socket joints, according to the original description; unfortunately the character state could not be confirmed by the authors after re-examining the paratypes specimens.

As for the clade "*Fujuriphyes*", it was supported by the combination of two apomorphies with homoplasy, namely the presence of ventrolateral setae (character 71) and the absence of ventromedial setae on segment 3 (character 79), which was only shared with *Pycnophyes faveolus* Brown, 1985. Additional apomorphies included the presence of ball and socket joints on segments 2-5 only (character 27; except for *Kinorhynchus distentus* Higgins, 1983 with ball and sockets also present on the segments 6-7) present also in three species in the clade "*Gymnophyes*" (a single additional gain), and the presence of ventrolateral setae and absence of ventromedial setae on segment 7 (characters 75, 84; except for *K. distentus*, with a reversion).

The clade "*Pycnophyes*" presented several apomorphies, although the characters behind these apomorphies showed a certain degree of homoplasy in other clades. "*Pycnophyes*" was supported by a combination of characters: presence of paradorsal setae on segments 4, 6 and 8, being absent on the remaining segments (characters 42-51), combined with the presence of middorsal elevations on all segments 2-9 (characters 32-39). The clade was furthermore supported by the following apomorphic character states (that were recovered convergently in other parts of the tree also): presence of groove-shaped dorsal and ventral cuticular scars along the trunk segments (characters 28-29); short lateral terminal spines when these are present (character 1); ventral sensory spots mesially located to the ventromedial setae on most segments (character 80). Despite *Pycnophyes norenburgi* Herranz et al., 2014 showed some differences from the remaining species of the clade, such as absence of paradorsal setae on segment 8, it shared most of the character previously referred. Even though *Kinorhynchus ilyocryptus* was only recovered as part of the clade "*Pycnophyes*" in one ML analysis its morphological characters were congruent with those of the clade.

ML analyses yielded two additional clades. The first one, named clade "*Krakenella*", formed by *Pycnophyes parasanjuanensis*, *P. sanjuanensis* and *Pycnophyes* sp. 2012b, was recovered sister to a large clade with "*Pycnophyes*", "*Gymnophyes*", *P. zelinkaei* and *Kinorhynchus ilyocryptus*. The second one, named "*Godzilliphyes*", with *K. apotomus*, *P. almansae*, *P. egyptensis*, *P. neuhausi*, *P. newguiniensis*, *P. newzealandiensis*, *P. robustus*, *Pycnophyes* sp. nov. 4 and *Pycnophyes* sp. 2012a. Excluding *Pycnophyes* sp. 2012a, the species of this clade shared unique combinations of characters (see Table 6), whereas *Pycnophyes* sp. 2012a had a very divergent morphology.

DISCUSSION

Phylogeny of Pycnophyidae

The monophyly of Pycnophyidae was fully supported in all our analyses, which was congruent with previous studies. The topologies obtained with the molecular and combined datasets were highly congruent under both dynamic homology and model-based methods, differing only in the position of *Kinorhynchus yushini*, *Pycnophyes greenlandicus* and *P. zelinkaei*. Maximum likelihood and Bayesian analyses on the total dataset yielded nine and seven clades within the family, respectively, each of them erected as new genera. Several of these new clades were relatively well-supported, despite the high amount of missing data. Exceptions were "*Planolimbus*", "*Higginsia*" and "*Cristaphyes*", which received lower nodal supports, but also accommodated most of the species represented only by the morphological partition.

The end of the traditional arrangement: conspicuous traits without phylogenetic information.

Neither *Pycnophyes* nor *Kinorhynchus* were monophyletic in any analyses, with the species of *Kinorhynchus* always recovered amongst *Pycnophyes* species. The potential paraphyly of both genera has previously been addressed by other authors (Higgins, 1962; Brown, 1985; Neuhaus, 1993; Lemburg, 2002). *Kinorhynchus* is solely distinguished from *Pycnophyes* by the absence of lateral terminal spines (Fig. 2i-j). However, *Kinorhynchus* has articulated bulbous protrusions at the same position as the lateral terminal spines in *Pycnophyes* (Fig. 2k-m). The protrusions are provided with thick cuticle, a central canal and a blunt terminal end (see Sánchez et al., 2014a). In fact, these bulbous structures resemble the developmental stages of the lateral terminal spines in *Pycnophyes*, *Paracentrophyes* and *Mixtophyes* (Neuhaus, 1993; Neuhaus, 1995; Lemburg, 2002; Sánchez et al., 2014a). This observation together with our results suggests that the alleged absence of lateral terminal spines in *Kinorhynchus* actually represents a rudimental stage of the structure, which has evolved convergently.

Taxonomic implications of the analysis: new classification of Pycnophyidae.

Neither our topologies nor the character tracing (with high homoplasy for the lateral terminal spines) supported the monophyly of *Kinorhynchus*, and Pycnophyidae was instead recovered as divided in nine clades. *Kinorhynchus* is hence synonymized with *Pycnophyes*, this latter retaining the priority and surviving redefined as one of the genera of the family. The remaining pycnophyid species were arranged in eight clades, all erected as new genera. Although two of these clades were recovered only by ML, they were supported by two unique combinations of characters. Despite the phylogenetic positions of *K. stenopygus*, *P. oshoroensis*, *P. tubuliferus*, *P. zelinkaei* and *Pycnophyes* sp. 2012a were unclear and they did not fit with the emended diagnosis of *Pycnophyes*, we assigned them to this genus tentatively, until new material or new phylogenetic studies resolve their positions. Hence, future researchers should be aware that the generic adscription of these five species is tentative, provisional and not derived from phylogenetic arguments. The combinations of characters that support the genera are summarized in Table 6. The old and the new names assigned after this work for each species are summarized in Table 7.

Pycnophyidae Zelinka, 1896 (emended)

Type genus: *Pycnophyes* Zelinka, 1907 (emended from Zelinka, 1907)

Genus composition: *Pycnophyes* n. comb., *Planolimbus* gen. nov., *Cristaphyes* gen. nov., *Higginsia* gen. nov., *Gymnophyes* gen. nov.,

Setaphyes gen. nov., *Fujuriphyes* gen. nov., *Krakenella* gen. nov. and *Godzilliphyes* gen. nov.

Emended diagnosis (from Higgins, 1990): Allomalorhagida with segment 1 consisting of one tergal plate, two episternal plates and a single midsternal plate; segments 2-11 with one tergal and two sternal plates; males with two pairs of long and flexible penile spines located between segment 10-11 and usually with a pair of large ventral tubes on segment 2; well-developed pachycycli, peg and socket joints, and apodemes (anteromesial thickenings of ventral pachycycli); seven placids: four dorsal and two or four ventral; fourteen trichoscalids (7 dorsal and 7 ventral) without trichoscalid plates; nine thin, long and flexible non-articulated outer oral styles. Spines and spinose processes are absent on all segments, whereas middorsal processes or elevations may be present. Cuticular setae may be present in various positions.

***Planolimbus* gen. nov.**

Type species (type by original designation): *Planolimbus pardosi* n. comb. (Sánchez et al., 2013).

Species composition: all species in the clade "*Planolimbus*" (*Planolimbus chalgap* n. comb. (Sánchez et al., 2013), *P. corrugatus* n. comb. (Higgins, 1983), *P. ecphantor* n. comb. (Higgins, 1983), *P. emarginatus* n. comb. (Higgins, 1983), *P. faveolus* n. comb. (Brown, 1999 in Adrianov and Malakhov 1999), *P. fimbriatus* n. comb. (Higgins, 1982), *P. lageria* n. comb. (Sánchez et al., 2014), *P. langi* n. comb. (Higgins, 1964), *P. mainensis* n. comb. (Blake, 1930), *P. pardosi* n. comb. (Sánchez et al., 2013), *P. sculptus* n. comb. (Lang, 1949)).

Diagnosis: Smooth posterior dorsal margin present on segments 7-10, absence of middorsal structure specializations; middorsal elevations may occur from the anterior segments until segment 6, otherwise with smooth posterior dorsal margin on all segments; laterodorsal setae often on each segment 2-9, sometimes absent on some segments; ventrolateral setae at least on segment 5; ventromedial setae often on segments 3-9, sometimes absent and substituted by ventrolateral or paraventral setae; well-developed pachycycli and peg and socket joints of similar sizes on segments 2-10, sometimes reduced on segments 8-10.

Etymology: From Latin *planus*, smooth, even + *limbus*, edge, border. Masculine gender.

***Cristaphyes* gen. nov.**

Type species (type by original designation): *Cristaphyes carinatus* n. comb. (Zelinka, 1928).

Species composition: all species in the clade "*Cristaphyes*" (*Cristaphyes abyssorum* n. comb. (Adrianov and Maiorova, 2015), *C. anomalus* n. comb. (Lang, 1953), *C. arctous* n. comb. (Adrianov, 1991 in Adrianov and Malakhov, 1991), *C. belizensis* n. comb. (Higgins, 1983), *C. carinatus* n. comb. (Zelinka, 1928), *C. chilensis* n. comb. (Lang, 1953), *C. chukchiensis* n. comb. (Higgins, 1991), *C. cristatus* n. comb. (Sánchez et al., 2013), *C. cryopygus* n. comb. (Higgins and Kristensen, 1988), *C. furugelmi* n. comb. (Adrianov, 1999 in Adrianov and Malakhov, 1999), *C. longicornis* n. comb. (Higgins, 1983), *C. nubilis* n. comb. (Sánchez et al., 2014), *C. odhneri* n. comb. (Lang, 1949), *C. phyllotropis* n. comb. (Brown and Higgins, 1983), *C. rabaulensis* n. comb. (Adrianov, 1999 in Adrianov and Malakhov, 1999), *C. spinosus* n. comb. (Lang, 1949), *C. yushini* n. comb. (Adrianov, 1989)).

Diagnosis: Pycnophyidae with middorsal processes surpassing the posterior margins of segments 2-9, often on segments 1 and 10 as well; middorsal process of segment 10 often well-developed, pointed tip, extending beyond the terminal trunk segment; sometimes keel-shaped middorsal processes on segments 1-10; middorsal processes of anterior segments often of similar sizes, turning progressively longer towards the posterior end; well-developed

pachycycli and peg and socket joints of similar sizes on segments 2-10, sometimes reduced on segment 10.

Etymology: From Latin *crista* crest, + Greek *phyes*, characterized by a form, the commonly used suffix in names of Allomalorhagid genera. The name makes reference to the conspicuous keel-shaped middorsal processes of trunk segments. Masculine gender.

***Higginsia* gen. nov.**

Type species (type by original designation): *Higginsia erismata* n. comb. (Higgins, 1983).

Species composition: all species in the clade "*Higginsia*" (*Higginsia cataphracta* n. comb. (Higgins, 1961), *H. dolichura* n. comb. (Sánchez et al., 2011), *H. erismata* n. comb. (Higgins, 1983), *H. trisetosa* n. comb. (Higgins, 1983)).

Diagnosis: Pycnophyidae with middorsal elevations never surpassing the posterior margin of segments 2-5; middorsal processes surpassing the posterior margin of any posterior segment (6-9), otherwise with middorsal elevations instead; paradorsal setae on segments 2, 4, 6 and 8 only, sometimes on segment 3; laterodorsal setae on segments 2-9, sometimes absent on segment 2; lateroventral setae on segments 2, 4, 6-8, sometimes on any segment 3, 5 or 9; ventrolateral setae at least on segments 5 and on any additional posterior segment (7-9); well-developed pachycycli and peg and socket joints of similar sizes on segments 2-10, sometimes reduced on segments 9-10.

Etymology: To honor Dr. Robert P. Higgins, main researcher on the phylum Kinorhyncha during the second half of the XX Century. Feminine gender.

***Gymnophyes* gen. nov.**

Type species (type by original designation): *Gymnophyes smaug* n. comb. (Sánchez et al., 2013).

Species composition: all species in the clade "*Gymnophyes*" (*Gymnophyes argentinensis* n. comb. (Martorelli and Higgins, 2004), *G. barentsi* n. comb. (Adrianov, 1999 in Adrianov and Malakhov, 1999), *G. borealis* n. comb. (Higgins and Korczynski, 1989), *G. canadensis* n. comb. (Higgins and Korczynski, 1989), *G. galtsovae* n. comb. (Adrianov, 1999 in Adrianov and Malakhov, 1999), *G. greenlandicus* n. comb. (Higgins and Kristensen, 1988), *G. farinellii* n. comb. (Sánchez et al., 2014), *G. maximus* n. comb. (Reimer, 1963), *G. mokievskii* n. comb. (Adrianov, 1995), *G. smaug* n. comb. (Sánchez et al., 2013), *G. spitsbergensis* n. comb. (Adrianov, 1995)).

Diagnosis: Very large Pycnophyidae, ranging from 800 µm up to 1 mm in total trunk length; middorsal elevations never surpassing the posterior margin of segments 2-9 (males of *G. galtsovae* with less conspicuous middorsal structure on the posterior segments); scarce in setae, paradorsal setae absent along the trunk (present only on segment 6 in *G. greenlandicus*); often with dot-shaped dorsal cuticular scars scattered on segments 2–10.

Etymology: From Greek *Gymnos*, bare, naked + *phyes*, characterized by a form, the commonly used suffix in names of Allomalorhagid genera. The name makes reference to the very few, if any, setae on the trunk segments. Masculine gender.

***Setaphyes* gen. nov.**

Type species (type by original designation): *Setaphyes dentatus* n. comb. (Reinhard, 1881).

Species composition: all species in the clade "*Setaphyes*" (*Setaphyes dentatus* n. comb. (Reinhard, 1881), *S. flaveolatus* n. comb. (Zelinka, 1928), *S. iniorhaptus* n. comb. (Higgins, 1983), *S. kielensis* n. comb. (Zelinka, 1928)) plus *S. australensis* n. comb. (Lemburg, 2002).

Diagnosis: Pycnophyidae with ventrolateral setae on segment 5, absent on segments 2-9; ball and socket joints reduced on the posterior segments, often well-developed on segments 2-3 only; scattered dot-shaped cuticular scars at both dorsal and ventral sides;

middorsal elevations never surpassing the posterior margin of segments 2-6; middorsal process surpassing the posterior margin of segments 7-9 may be present, otherwise with middorsal elevations instead; paired or unpaired paradorsal setae on segments 2-9; lateroventral setae on segments 2-10.

Etymology: From Latin *Seta*, seta, hair + Greek *phyes*, characterized by a form, the commonly used suffix in names of Allomalorhagid genera. The name makes reference to the abundant setae present on the trunk segments. Masculine gender.

Remarks: *Setaphyes australensis* is provisionally assigned to this genus since it shares many of its diagnostic features, and only differs in the distribution of pachycycli with ball and socket joints.

***Fujuriphyes* gen. nov.**

Type species (type by original designation): *Fujuriphyes ponticus* n. comb. (Zelinka, 1928).

Species composition: all species in the clade "*Fujuriphyes*" (*Fujuriphyes deirophorus* n. comb. (Higgins, 1983), *F. distentus* n. comb. (Higgins, 1983), *F. ponticus* n. comb. (Zelinka, 1928), *F. rugosus* n. comb. (Zelinka, 1928), *Fujuriphyes* sp. nov. 5).

Diagnosis: Pycnophyidae with ventrolateral setae on segment 5 and on additional segments, from segments 3-9; ventromedial setae absent on the segments where the ventrolateral setae are present, except for segment 5 with both ventromedial and ventrolateral setae present; ball and socket joints reduced on the posterior segments, often well-developed on segments 2-5 only; middorsal elevations never surpassing the posterior margin of segments 2-9; paradorsal setae on segments 2, 4, 6 and 8 only; laterodorsal setae on segments 2-9; lateroventral setae on segments 2, 4, 6, 8 and 10 only; ventral setae not longitudinally aligned along the trunk segments; when present, lateral terminal spines are long, with a lateral terminal spines/total trunk length proportion of >30%; often without ventral tubes on segment 2 in males.

Etymology: From *Fujur*, the dog-dragon in the novel "The Never-ending Story" by M. Ende + Greek *phyes*, characterized by a form, the commonly used suffix in names of Allomalorhagid genera. The name adds to the list of kinorhynch (mud dragons) species named after dragons and also refers to the study of kinorhynch phylogeny as a "never-ending story". Masculine gender.

***Krakenella* gen. nov.**

Type species (type by original designation): *Krakenella parasanjuanensis* n. comb. (Adrianov and Higgins, 1996).

Species composition: *Krakenella parasanjuanensis* n. comb. (Adrianov and Higgins, 1996), *K. sanjuanensis* n. comb. (Higgins, 1961), *Krakenella* sp. 2012b.

Diagnosis: Pycnophyidae with large size, with a total trunk length ranging from 700 µm up to 1 mm; lateroventral setae on segments 2, 4, 6-8, 10 only; middorsal elevations that never surpass the posterior margin of all segments 2-9, flanked by paradorsal setae on each segment; laterodorsal setae on segments 2-9; ventrolateral setae on segment 5; often with rounded-oval dorsal and ventral cuticular scars.

Etymology: From *Kraken*, marine monster of the Scandinavian mythology + Latin *–ella* diminutive suffix. Feminine gender.

Remarks: *Krakenella* gen. nov. is erected for *K. parasanjuanensis*, *K. sanjuanensis* and *Krakenella* sp. 2012b as these species were recovered as a clade in ML analyses of the total dataset, sister to the clades "*Pycnophyes*", "*Planolimbus*", *Pycnophyes zelinkaei* and *Kinorhynchus ilyocryptus*, and it can be unambiguously diagnosed by a combination of characters (see Table 6).

***Godzilliphyes* gen. nov.**

Type species: *Godzilliphyes robustus* n. comb. (Zelinka, 1928).

Species composition: *G. almansae* n. comb. (Sánchez et al., 2014), *G. apotomus* n. comb. (Higgins, 1983), *G. egyptensis* n. comb. (Higgins, 1966), *G. neuhausi* n. comb. (Higgins, 2004 in Martorelli and Higgins, 2004), *G. newguiniensis* n. comb. (Adrianov, 1999 in Adrianov and Malakhov, 1999), *G. newzealandiensis* n. comb. (Adrianov, 1999 in Adrianov and Malakhov, 1999), *Godzilliphyes robustus* n. comb. (Zelinka, 1928), *Godzilliphyes* sp. nov. 4.

Diagnosis: Pycnophyidae with middorsal elevations never surpassing the posterior margin of segments 2-9; paradorsal setae on segments 2-9; laterodorsal setae on segments 2-8, sometimes on segment 9; lateroventral setae at least on segments 2, 4, 6-8, absent on segment 5; ventrolateral setae present on segment 5; often with groove-shaped dorsal and ventral cuticular scars.

Etymology: From *Godzilla*, marine dinosaur-like monster originally from Japanese movies + Greek *phyes*, characterized by a form, the commonly used suffix in names of Allomalorhagid genera. Masculine gender.

Remarks: ML analyses recovered a clade with all the species of *Godzilliphyes* together with *Pycnophyes* 2012a, which is not assigned to *Godzilliphyes* as it presents a very divergent morphology.

***Pycnophyes* Zelinka, 1907 (emended)**

Type species: *Pycnophyes communis* Zelinka, 1908.

Species composition: all species in the clade "*Pycnophyes*" (*Pycnophyes aulacodes* Sánchez et al., 2011, *P. beaufortensis* Higgins, 1964, *P. calmani* Southern, 1914, *Pycnophyes communis* (Zelinka, 1908), *P. frequens* Blake, 1930, *P. giganteus* n. comb. (Zelinka, 1908), *P. norenburgi* Herranz et al., 2014, *P. paraneapolitanus* n. comb. (Sheremetevskij, 1974), *P. schornikovi* Adrianov, 1999 in Adrianov and Malakhov, 1999) plus *P. ilyocryptus* n. comb. (Higgins, 1961), *P. oshoroensis* Yamasaki et al., 2012, *P. stenopygus* n. comb. (Higgins, 1983), *P. tubuliferus* Adrianov, 1989, *P. zelinkaei* Southern, 1914 and *Pycnophyes* sp. 2012a.

Diagnosis (emended from Adrianov and Malakhov, 1999): Pycnophyidae with middorsal elevations that never surpass the posterior margin of segments 2–9; paired or unpaired paradorsal setae on segments 4, 6, and 8 only; laterodorsal setae on segments 2–9; lateroventral setae on segments 2, 4, 6 and 8, absent on uneven segments except for the segment 9 where they may be present; ventrolateral setae on segment 5 only; ventromedial setae on segments 3–9; intracuticular pores along the trunk surface; when present lateral terminal spines are short, with a lateral terminal spines/total trunk length proportion of <20%; often with groove-shaped dorsal and ventral cuticular scars along the trunk, midventral midsternal projection on segment 1 and ventral sensory spots mesially located to the ventromedial setae on most segments; well-developed pachycycli and peg and socket joints of similar sizes on segments 2-10, sometimes reduced on segments 9 and 10.

Remarks: Despite *Pycnophyes norenburgi* differs from the remaining species of the genus in the absence of paradorsal setae on segment 8 and presence of laterodorsal setae on segment 9 in males only, we keep the species within this genus until new research has been carried out. The morphological characters of *Pycnophyes ilyocryptus* were congruent with the emended diagnosis and apomorphies of *Pycnophyes* in our analyses. Therefore the species is provisionally assigned to this genus.

Kinorhynchus is synonymized with *Pycnophyes* (see above). Despite *P. oshoroensis*, *P. stenopygus*, *P. tubuliferus*, *P. zelinkaei* and *Pycnophyes* sp. 2012a did not fit the emended diagnosis of *Pycnophyes* they are tentatively assigned to the type genus of the family

Table 6. Summary of combination of characters that define the genera. Abbreviations: LTS, lateral terminal spines; LV, lateroventral; TL, total trunk length, VL, ventrolateral.

Structure	Character number	*Pycnophyes*	*Gymnophyes* gen. nov.	*Planolimbus* gen. nov.	*Cristaphyes* gen. nov.	*Higginsia* gen. nov.	*Setaphyes* gen. nov.	*Fujuriphyes* gen. nov.	*Krakenella* gen. nov.	*Godzilliphyes* gen. nov.
Middorsal specialization	32-39	middorsal elevations 2-9	middorsal elevation 2-9	smooth posterior dorsal margin 7-10	middorsal processes 2-9, sometimes on 10	middorsal elvations 2-5, middorsal process on some 6-9	middorsal elevation 2-6, sometimes on 7-9	middorsal elevations 2-9	middorsal elevations 2-9	middorsal elevations 2-9
PD Setae	42-51	4, 6, 8 only	absent, if present on a single segment	-	-	2, 4, 6, 8, sometimes on 3	all 2-9	2, 4, 6, 8 only	all 2-9	all 2-9
LV setae	61-67	always on 2, 4, 6, 8; absent on segments 3, 5, 7	-	-	-	always on segment 2, 4,6-8	all 2-10	always on 2, 4, 6, 8; absent on 3, 5, 7	always on 2, 4, 6-8, absent on 3, 5, 9	always on 2, 4, 6-8; absent on 5
VL setae (excluding segment 1, 2, 10)	71-77, 79, 81-84, 86, 87	present on 5 only	often on 5	often on 5	present on 5	present on 5 and in one segment on 7-9	absent on all segments 2-9	present on 5 and at least on two segments 3-4, 6-9 without ventromedial setae	present on segment 5	present on segment 5
Pachycycli	27	-	-	-	-	-	2-3 only	2-5 or 2-7 only	-	-
Cuticular scars	28, 29	groove-shaped dorsally and often ventrally	often scattered dot-shaped dorsally	-	-	rounded-oval shaped dorsally	scattered dot-shaped dorsal and ventrally	rounded-oval shaped dorsal and ventrally	rounded-oval ventral and dorsally	often groove-shaped dorsal and ventrally
Spines (LTS/TL)	1	<20%	-	-	-	-	-	>30%	-	-
Trunk lenght	4	-	>800 µm	-	-	-	-	-	>700 µm	-

(see above). *Pycnophyes zelinkaei* and *Pycnophyes* sp. 2012a had several autapomorphies and very divergent morphologies leaving their phylogenetic affinities as uncertain; whereas *Pycnophyes oshoroensis*, *P. stenopygus* and *P. tubuliferus* were recovered together in some of our analyses, but none unique combination of characters was found.

Morphological character evolution

Inclusion of the morphological partition.

The high percentage of missing data after the inclusion of those pycnophyids represented only by morphology resulted in lower nodal supports and polytomies in our topologies, which was a consequence of the rogue behavior of some terminals (Giribet et al., 2012; Martínez et al., 2014). However, it also provided additional information and better understanding of the evolution of the family. Total evidence analyses helped to resolve the position of *Cristaphyes yushini*, the most unstable terminal in the molecular and combined analyses. In the total evidence analysis *C. yushini* was unambiguously recovered in a large, well-supported clade together with 31 species, none of them represented by molecular data. The instability of this species was likely related to insufficient taxon sampling. This may also be the case of *Pycnophyes zelinkaei*, with very divergent morphology, which was recovered in a large clade together with "*Pycnophyes*", "*Gymnophyes*", *Pycnophyes ilyocryptus* and *Pycnophyes* sp. 2012b. Discovery of new taxa might provide further information allowing the placement of this species as well.

Morphology also revealed several large clades of taxa that were not included in our molecular datasets. These clades were recovered with high nodal support and defined by several unique apomorphies, showing novel aspects of the evolution of Pycnophyidae, which open new questions and warrant further research. Some of these clades showed unique combinations of characters, such for *Planolimbus* n. gen, defined by the absence of middorsal specializations, and *Gymnophyes* gen. nov., which was supported by the absence of middorsal setae and a long trunk length.

Moreover, morphology compensated the geographical bias of our molecular dataset, mostly

consisting of European and East Asian species due to the major sampling effort performed in these two areas (see Table 1). This is a common problem not only to many phylogenetic analyses, but also to more general taxonomic studies, and might confound the actual biogeographic patterns in certain groups (Curini-Galletti et al., 2012), or generate patterns strongly biased by the sampling effort. In our case, inclusion of all described species revealed an entire clade dominated by deep-sea and Artic species (see below), only represented by *Gymnophyes greenlandicus* in our molecular dataset.

Lastly, assuming the methodological problems, our total dataset also provided significant aspects of the character evolution within the group and allowed us for testing morphological hypothesis proposed in previous morphological studies.

Recursive loss of ventral tubes in males.

The presence of male-specific ventral tubes on segment 2 was apomorphic for Pycnophyidae (Sørensen et al., in press), although they were absent in some species (Neuhaus, 2013; Sánchez et al., 2014b). The paired ventromedial tubes might have a secretive function, possibly involved in reproduction (see Fig. 2h), and they have traditionally been referred to as adhesive tubes (Zelinka, 1928; Higgins, 1983; Kristensen and Higgins, 1991). Species of Neocentrophyidae lack tubes on segment 2, whereas species of *Dracoderes*, *Franciscideres* and several cyclorhagid genera, including *Echinoderes*, also bear a pair of tubes on segment 2, but they are present in both sexes and in a different position, and might therefore not be homologous to those in Pycnophyidae (Sørensen and Pardos, 2008; Sørensen et al., 2012; Dal Zotto el al., 2013; Thomsen et al., 2013).

Recently, Sánchez et al (2014b) highlighted the absence of ventral tubes on segment 2 in *Cristatus longicornis*, *Cristatus chilensis*, *Fujuriphyes rugosus*, *Fujuriphyes ponticus*, *Fujuriphyes* sp. nov. 5, *Godzilliphyes egyptensis*, *Godzilliphyes* sp. nov. 4, *Gymnophyes farinellii*, *Higginsia dolichurus*, and *Planolimbus ecphantor*. This species also shared the presence of long lateral terminal spines (LTS/total trunk length>30%), another uncommon trait in the family, which together suggest that these species might be closely related. However, these ten species were not recovered together in our analyses, which instead showed several independent losses of tubes in males and gain of large lateral terminal spines in the family.

Middorsal structure specializations.

Middorsal structure specializations on the posterior margin of trunk segments, including spines, spinose processes, processes and elevations, have been commonly used as taxonomic characters in Kinorhyncha (see Fig. 1). They all consist of hollow structures with rigid walls and closed tips, differing among them in their length and morphology. Middorsal spines, spinose processes and processes are all cuticular protrusions. However, while spines are rigid and articulated at the basis, middorsal spinose processes are flexible and non-articulated, and processes are non-articulated and rigid. Middorsal elevations are inconspicuous short cuticular structures (see Appendix *Character description*).

Spines are present in all Cyclorhagida, whereas their occurrence is scarcer amongst the Allomalorhagida species. Franciscideridae and Dracoderidae have spines on several segments, whereas all Neocentrophyidae bear spines on the last trunk segments and middorsal spinose processes on the remaining ones (Fig. 1b, g). Besides the lateral terminal ones, spines are never present in Pycnophyidae. Instead these species are equipped with middorsal processes, middorsal elevations or no middorsal specializations at all (Fig. 1c-e, h-o). Neuhaus (1993, 2013) suggested a homology between

the middorsal spines and middorsal spinose processes in Neocentrophyidae. The middorsal structure specializations present in the earlier juvenile stages of Neocentrophyidae (middorsal processes, Sánchez pers. obs.) give rise through subsequent molts to middorsal spinose processes on the anterior segments of the adult and middorsal spines on the posterior ones (Sánchez pers. obs.; Neuhaus, 1995). Moreover, middorsal specializations are also present in juvenile stages of Pycnophyidae (Fig. 1f) (middorsal processes, Sánchez pers. obs.), giving rise to processes, elevations or disappearing in the adults (Sánchez pers. obs.; Brown, 1985; Higgins and Kristensen, 1988; Neuhaus, 1993; Lemburg, 2002). These observations, together with our character tracing suggested the homology of these structures, with a transformation series from the middorsal spines (plesiomorphic condition of Allomalorhagida, which can occur on any segment from segment 1 to 11 according to Sørensen et al., in press) into middorsal spinose processes on the anterior segments in Neocentrophyidae and middorsal elevations on all segments in Pycnophyidae, both as apomorphies for the families. Within Pycnophyidae, middorsal elevations were lost in *Planolimbus* gen. nov. (Fig. 1i-k, o) or transformed into middorsal processes in *Cristaphyes* gen. nov. (Fig. 1h), as well as on certain segments of *Higginsia* gen. nov., *Setaphyes dentatus* and *S. flaveolatus* (Fig. 1l) (see Fig. 5).

Since spines, middorsal spinose processes and processes are present in all juveniles of Allomalorhagida, their presence in adults of Neocentrophyidae and Pycnophyidae might represent a retention of juvenile characters. Neocentrophyidae always have middorsal spinose processes on segments 1-9 and spines on segments 10-11 in males or 11 in females (Fig. 1b). In Pycnophyidae, the species of *Cristaphyes* gen. nov. have middorsal processes on segments 1-9 and sometimes also on 10 (Fig. 1c, h), whereas those of *Higginsia* gen. nov., *Setaphyes dentatus* and *S. flaveolatus* have middorsal processes on segments 7-9, and middorsal elevation on segments 1-6 (Fig. 1 l-m) (see Fig. 5). The retention of juvenile states might be more general in some of these species, since in the adults of *Cristaphyes* gen. nov. (Fig. 1c, h) the length of middorsal processes increases towards the posterior end, as in the juveniles of all kinorhynchs (Fig. 1f) (Brown, 1985; Neuhaus, 1993, 1995, 2013; Higgins and Kristensen, 1988; Lemburg, 2002).

Distribution of setae along the trunk.

Except for *Cristaphyes carinatus* and *Higginsia trisetosus*, all Pycnophyidae species present a single pair of ventral setae on segments 3-4 and 6-9, aligned as a row along the trunk in ventromedial or ventrolateral position. Two pairs are only present on segment 5, one in ventrolateral and one in ventromedial position. This distribution of setae represented the plesiomorphic condition of the family and it was retained in most species. Setae distributions on segments 2 and 10 were more variable and depended on sexual dimorphism or yielded ambiguous character optimizations.

The optimization of the ventrolateral setae patterns in Pycnophyidae yielded presence of ventrolateral setae on segment 5 and absence on segments 3-4 and 6-9 as plesiomorphic condition of the family. The ventrolateral setae on segment 5 were lost ones in *Setaphyes* gen. nov., *Planolimbus lageria* and *Gymnophyes argentinensis*, whereas the ventrolateral setae on the remaining segments were convergently gained in *Fujuriphyes* gen. nov. and as apomorphic of few species.

Distribution of sensory spots.

Kinorhynch have three types of sensory spots, present in both adults and juvenile stages (Fig. 2d-f) (Neuhaus, 1993; Lemburg, 2002; Sørensen et al., 2010b), although they are rarely reported in the old literature because they are hard to visualize with light microscopy. The plesiomorphic condition for

Pycnophyidae was the presence of one ventral pair of sensory spots on segments 3-9, either of type 1 or 2 (Fig. 2f), located lateral to the ventromedial setae. This number of sensory spots, except for that on segment 9, was retained in most of the species. On segment 9, the number of sensory spots showed several transformation series, with transformations from one pair to two pairs in many species, and from two pairs into three pairs in *Cristaphyes phyllotropis*, in the subclade rooted by *Planolimbus mainensis* and in "*Pycnophyes paraneapolitanus-Pycnophyes communis*". The position of the sensory spots shifted convergently from mesial to lateral in relation to the ventromedial seta in the genus *Pycnophyes* (Fig. 2f) and in the well-supported subclade "*Cristaphyes cristatus-C. furugelmi"* (see below).

Pachycycli and ball and socket joints.

The presence of pachycycli with well-developed ball and socket joints articulating the dorsal and tergal plates on segments 2-10 (see Fig. 2a and Appendix *Character description*) was a unique apomorphy of Pycnophyidae. The ball and socket joints were reduced convergently on segments 8-10 of several species. The reduction of ball and socket joints was less homoplastic towards the anterior segments, being retained independently on segments 2-5 twice in the subclade "*Gymnophyes barentsi-G. mokievskii"* and in *Fujuriphyes* gen. nov. (except for *F. distentus*, which had ball and socket joints on segments 2-7) and retained on segments 2-3 only three times independently in *Setaphyes* gen. nov., *Gymnophyes spitsbergensis* and in "*Godzilliphyes almansae-Godzilliphyes robustus*".

Large species in cold waters.

As for many other meiofaunal groups (Worsaae, 2005; Curini-Galletti et al., 2012; Di Domenico et al., 2014; Martínez et al., 2014; Scarpa et al., 2015), most of the clades of our analyses are globally distributed, and only few of them exhibited different biogeographical pattern. In particular, *Gymnophyes* gen. nov., seven species in *Cristaphyes* gen. nov. and three additional species of Pycnophyidae are exclusive from cold waters, either in the deep-sea or in high latitudes (Adrianov and Malakhov, 1999; Neuhaus, 2013; Sánchez et al, 2014b). These species share a long trunk compared to other tropical or temperate species included in the analyses. The plesiomorphic total trunk length in Pycnophyidae was 600-700 µm that evolved into longer trunks in species of cold waters, with an increase to 800-1000 µm recovered in these two groups. In addition, several deep-sea species (100-5000 m depth) were recovered in a highly supported clade (BPP: 0.99) within *Cristaphyes* gen. nov., and they shared the presence of a conspicuous keel-shaped middorsal processes along all trunk segments as a unique apomorphic character. The keel-shaped middorsal processes increase in length towards the posterior segments, becoming a prominent keel on segment 10 that overlaps the first half of the segment 11 as another unique apomorphy for the group (Fig. 1c).

Evaluation of other characters used in the taxonomy of Pycnophyidae.

Our analyses confirmed that several morphological characters frequently used in the taxonomy of Pycnophyidae were very homoplastic within our clades, and therefore useful for species identification due to its interspecific variability. These characters included the distribution and number of paradorsal setae by each segment; the distribution of laterodorsal setae, often on segments 2-9 (their absence on any segment turns in a highly recognizable character of a particular species); the shape of the dorsal and ventral cuticular scars (even though the rounded-oval plesiomorphic condition was widespread distributed, the presence of groove-shaped was a distinctive trait in most species of *Godzilliphyes* gen. nov. and

Before this study	This study
K. anomalus Lang, 1953	*Cristaphyes anomalus* n. comb. (Lang, 1953)
K. apotomus Higgins, 1983	*Godzilliphyes apotomus* n. comb. (Higgins, 1983)
K. belizensis Higgins, 1983	*Cristaphyes belizensis* n. comb. (Higgins, 1983)
K. cataphractus Higgins, 1961	*Higginsia cataphracta* n. comb. (Higgins, 1961)
K. deirophorus Higgins, 1983	*Fujuriphyes deirophorus* n. comb. (Higgins, 1983)
K. distentus Higgins, 1983	*Fujuriphyes distentus* n. comb. (Higgins, 1983)
K. erismatus Higgins, 1983	*Higginsia erismata* n. comb. (Higgins, 1983)
K. fimbriatus Higgins, 1982	*Planolimbus fimbriatus* n. comb. (Higgins, 1982)
K. giganteus Zelinka, 1928	*Pycnophyes giganteus* n. comb. (Zelinka, 1908)
K. ilyocryptus Higgins, 1961	*Pycnophyes ilyocryptus* Higgins, 1961
K. langi Higgins, 1964	*Planolimbus langi* n. comb. (Higgins, 1964)
K. mainensis Blake, 1930	*Planolimbus mainensis* n. comb. (Blake, 1930)
K. paraneapolitanus Sheremetevskij, 1974	*Pycnophyes paraneapolitanus* n. comb. (Sheremetevskij, 1974)
K. phyllotropis Brown and Higgins, 1983	*Cristaphyes phyllotropis* n. comb. (Brown and Higgins, 1983)
K. rabaulensis Adrianov 1999	*Cristaphyes rabaulensis* n. comb. (Adrianov, 1999 in Adrianov and Malakhov, 1999)
K. spinosus Lang, 1949	*Cristaphyes spinosus* n. comb. (Lang, 1949)
K. stenopygus Higgins, 1983	*Pycnophyes stenopygus* Higgins, 1983
K. trisetosus Higgins, 1983	*Higginsia trisetosa* n. comb. (Higgins, 1983)
K. yushini Adrianov, 1989	*Cristaphyes yushini* n. comb. (Adrianov, 1989)
P. sp. 2012a	*Pycnophyes* 2012a
P. sp. 2012b	*Krakenella* 2012b
P. abyssorum Adrianov and Maiorova, 2015	*Cristaphyes abyssorum* n. comb. (Adrianov and Maiorova, 2015)
P. almansae Sánchez et al., 2014	*Godzilliphyes almansae* n. comb. (Sánchez et al., 2014)
P. arctous Adrianov, 1999	*Cristaphyes arctous* n. comb. (Adrianov, 1991 in Adrianov and Malakhov, 1991)
P. argentinensis Martorelli and Higgins, 2004	*Gymnophyes argentinensis* n. comb. (Martorelli and Higgins, 2004)
P. aulacodes Sánchez et al., 2011	*Pycnophyes aulacodes* Sánchez et al., 2011
P. australensis Lemburg, 2002	*Setaphyes australensis* n. comb. (Lemburg, 2002)
P. barentsi Adrianov, 1999	*Gymnophyes barentsi* n. comb. (Adrianov, 1999 in Adrianov and Malakhov, 1999)
P. beaufortensis Higgins, 1964	*Pycnophyes beaufortensis* Higgins, 1964
P. borealis Higgins and Korczynski, 1989	*Gymnophyes borealis* n. comb. (Higgins and Korczynski, 1989)
P. calmani Southern, 1914	*Pycnophyes calmani* Southern, 1914
P. canadensis Higgins and Korczynski, 1989	*Gymnophyes canadensis* n. comb. (Higgins and Korczynski, 1989)
P.carinatus Zelinka, 1928	*Cristaphyes carinatus* n. comb. (Zelinka, 1928)
P. chalgap Sánchez et al., 2013	*Planolimbus chalgap* n. comb. (Sánchez et al., 2013)
P. chilensis Lang, 1953	*Cristaphyes chilensis* n. comb. (Lang, 1953)
P. chukchiensis Higgins, 1991	*Cristaphyes chukchiensis* n. comb. (Higgins, 1991)
P. communis Zelinka, 1908	*Pycnophyes communis* Zelinka, 1908
P. corrugatus Higgins, 1983	*Planolimbus corrugatus* n. comb. (Higgins, 1983)
P. cristatus Sánchez et al., 2013	*Cristaphyes cristatus* n. comb. (Sánchez et al., 2013)
P. cryopygus Higgins and Kristensen, 1988	*Cristaphyes cryopygus* n. comb. (Higgins and Kristensen, 1988)
P. dentatus Reinhard, 1881	*Setaphyes dentatus* n. comb. (Reinhard, 1881)
P. dolichurus Sánchez et al., 2011	*Higginsia dolichura* n. comb. (Sánchez et al., 2010)
P. ecphantor Higgins, 1983	*Planolimbus ecphantor* n. comb. (Higgins, 1983)
P. egyptensis Higgins, 1966	*Godzilliphyes egyptensis* n. comb. (Higgins, 1966)
P. emarginatus Higgins, 1983	*Planolimbus emarginatus* n. comb. (Higgins, 1983)
P. farinellii Sánchez et al., 2014	*Gymnophyes farinellii* n. comb. (Sánchez et al., 2014)
P. faveolus Brown, 1985	*Planolimbus faveolus* n. comb. (Brown, 1999 in Adrianov and Malakhov, 1999)
P. flaveolatus Zelinka, 1928	*Setaphyes flaveolatus* n. comb. (Zelinka, 1928)
P. frequens Blake, 1930	*Pycnophyes frequens* Blake, 1930
P. furugelmi Adrianov, 1999	*Cristaphyes furugelmi* n. comb. (Adrianov, 1999 in Adrianov and Malakhov, 1999)
P. galtsovae Adrianov, 1999	*Gymnophyes galtsovae* n. comb. (Adrianov, 1999 in Adrianov and Malakhov, 1999)
P. greenlandicus Higgins and Kristensen, 1988	*Gymnophyes greenlandicus* n. comb. (Higgins and Kristensen, 1988)
P. iniorhaptus Higgins, 1983	*Setaphyes iniorhaptus* n. comb. (Higgins, 1983)
P. kielensis Zelinka, 1928	*Setaphyes kielensis* n. comb. (Zelinka, 1928)
P. lageria Sánchez et al., 2014	*Planolimbus lageria* n. comb. (Sánchez et al., 2014)
P. longicornis Higgins, 1983	*Cristaphyes longicornis* n. comb. (Higgins, 1983)
P. maximus Reimer, 1963	*Gymnophyes maximus* n. comb. (Reimer, 1963)
P. mokievskii Adrianov, 1995	*Gymnophyes mokievskii* n. comb. (Adrianov, 1995)
P. neuhausi Martorelli and Higgins, 2004	*Godzilliphyes neuhausi* n. comb. (Higgins, 2004 in Martorelli and Higgins, 2004)
P. newguiniensis Adrianov, 1999	*Godzilliphyes newguiniensis* n. comb. (Adrianov, 1999 in Adrianov and Malakhov, 1999)

P. newzealandiensis Adrianov, 1999	*Godzilliphyes newzealandiensis* n. comb. (Adrianov, 1999 in Adrianov and Malakhov, 1999)
P. norenburgi Herranz et al., 2014	*Pycnophyes norenburgi* Herranz et al., 2014
P. nubilis Sánchez et al., 2014	*Cristaphyes nubilis* n. comb. (Sánchez et al., 2014)
P. odhneri Lang, 1949	*Cristaphyes odhneri* n. comb. (Lang, 1949)
P. oshoroensis Yamasaki et al., 2012	*Pycnophyes oshoroensis* Yamasaki et al., 2012
P. parasanjuanensis Adrianov and Higgins, 1996	*Krakenella parasanjuanensis* n. comb. (Adrianov and Higgins, 1996)
P. pardosi Sánchez et al., 2013	*Planolimbus pardosi* n. comb. (Sánchez et al., 2013)
P. ponticus Zelinka, 1928	*Fujuriphyes ponticus* n. comb. (Zelinka, 1928)
P. robustus Zelinka, 1928	*Godzilliphyes robustus* n. comb. (Zelinka, 1928)
P. rugosus Zelinka, 1928	*Fujuriphyes rugosus* n. comb. (Zelinka, 1928)
P. sanjuanensis Higgins, 1961	*Krakenella sanjuanensis* n. comb. (Higgins, 1961)
P. schornikovi Adrianov, 1999	*Pycnophyes schornikovi* Adrianov, 1999 in Adrianov and Malakhov, 1999
P. sculptus Lang, 1949	*Planolimbus sculptus* n. comb. (Lang, 1949)
P. smaug Sánchez et al., 2013	*Gymnophyes smaug* n. comb. (Sánchez et al., 2013)
*P.*sp. nov. 4	*Fujuriphyes* sp nov 4
*P.*sp. nov. 5	*Krakenella* sp. nov. 5
P. spitsbergensis Adrianov, 1995	*Gymnophyes spitsbergensis* n. comb. (Adrianov, 1995)
P. tubuliferus Adrianov, 1989	*Pycnophyes tubuliferus* Adrianov, 1989
P. zelinkaei Southern, 1914	*Pycnophyes zelinkaei* Southern, 1914

Pycnophyes n. comb. (Fig. 2b) as well as the exclusive presence of scattered dot-shaped in *Gymnophyes* gen. nov. and *Setaphyes* gen. nov. (Fig. 2c)); the presence/absence of midventral projection on segment 1 (Fig. 2g-h); the presence of longitudinal thickening on segment 10 as well as the presence of dagger-shaped structures on segment 9 are uncommon but conspicuous features, so the species that bear some of these features are easily recognizable.

Therefore we encourage the inclusion of these traits in future species descriptions, as it is being done, as well as those characters with phylogenetic information previously referred. Moreover, we suggest the addition of some other characters in the description of species in order to have more data to code and trace the character reconstruction in future studies. These characters are as follows: information on juvenile stages, shape of the anterior dorsal margin (smooth/denticulated), ornamentation in the anterior dorsal area of segment 1 (i.e. reticulated and covering a wide or broad area/ornamentation as circlets), ornamentation on the lateral terminal spines, presence of cuticular ornamental wrinkles in the anteriormost region of the segments, presence and number of cuticular ridges by segment, presence of intracuticular pores along the surface.

Overall diversity of Kinorhyncha

The total number of valid kinorhynch species has increased considerably during the last years, currently exceeding the 200 described species (Sørensen, 2013). Most of these species were collected along the American East coast, Caribbean Sea, Artic coast of Russia, European West coast and around the Korean, the Iberian and the Italian Peninsula (Zelinka, 1928; Higgins, 1983; Adrianov and Malakhov, 1999; Landers et al., 2012; Sánchez et al., 2012, 2013, 2014c), showing an apparently high kinorhynch diversity. Similar studies in other regions would determine whether these areas are really hot spots for kinorhynchs or new surveys in other localities around the world would yield comparable numbers of new species. The diversity patterns in kinorhynchs derived from our present data correspond better to sampling distribution than to kinorhynch distribution. This fact would evidence that the diversity and biogeography of kinorhynchs is still far away from being known.

CONCLUSIONS

The paraphyly of *Pycnophyes* and *Kinorhynchus* was corroborated by total evidence analysis and the genera can therefore no longer be considered as natural groups. Accordingly, *Pycnophyes* and *Kinorhynchus* were rejected as a taxonomic and phylogenetic units and a new classification of Pycnophyidae was proposed. We accommodate all the described species of Pycnophyidae into nine genera, each corresponding to a clade supported by molecular and morphological characters, either by unique apomorphies or combination of characters. As for the treatment of the molecular sequences, the deletion of all gaps did not significantly affect the phylogenetic reconstruction since both dataset yielded the same tree topology and with similar nodal support for each clade. Lastly, the inclusion of the 64 species only represented by the morphological partition allowed us to compensate the molecular sampling bias, to resolve the position of some unstable species and to trace the morphological character evolution with phylogenetic value.

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RESULTS

Appendix I

Morphology

I.I. KINORHYNCHS THROUGH MICRO-CT (KINORHYNCHA: ALLOMALORHAGIDA): A NEW WINDOW INTO THE MEIO-WORLD

N. Sánchez and J. Alba-Tercedor

INTRODUCTION

Since the beginnings of Zoology, the study of the anatomy of little animals has been a challenge for researchers who tried to develop many different techniques to observe the internal organs and structures in a non-destructive way. Nowadays, with the development of micro-CT, we have a very "attractive" and useful tool for that purpose. We have applied it to several small invertebrate groups, such as Opisthobranchia, Coleoptera, Ephemeroptera or Diptera. Following with this line we decided to go a step further and investigate a poorly known group of even smaller animals, the kinorhynchs or mud dragons.

Kinorhyncha comprises a phylum of exclusively marine, benthic, free-living, meiofaunal animals of 0.13–1.04 mm body length. Their body is composed of a retractable introvert, neck, and 11 trunk segments, with many segmentally arranged internal and external organs, including the cuticle, spines, glands, sensory spots, muscles and nerve components. Although the phylum was discovered more than 150 years ago, only a small group of researchers have paid attention to them, and hence its study is still on a pioneer stage at all levels, including that on internal anatomy.

Detailed works on this matter were not available until the use of Transmission Electron Microscopy (TEM) at the end of the twenty century (Kristensen and Higgins, 1991). However, this technique implies the total destruction of the animal. Recently, different studies through Confocal Laser Scanning Microscopy (CLSM), mostly for Cyclorhagida (Müller and Schmidt-Rhaesa, 2003; Rothe and Schmidt-Rhaesa, 2004; Schmidt-Rhaesa and Rothe, 2006; Herranz et al., 2013, 2014a), have been made in order to improve our knowledge on different kinorhynch organ systems, but unfortunately this technique requires freshly captured animals.

As for potential alternative for these both techniques, we present herein a non-destructive imaging technique for the first time in kinorhynchs using already fixed homalorhagid specimens.

MATERIAL AND METHODS

Specimens of *Pycnophyes robustus* Zelinka, 1928 were found in samples from Ceuta (22/05/2013), and extracted from the sediment using the bubbling technique of Higgins (Sørensen and Pardos, 2008). Specimens were fixed in 100% ethanol before the stained with 1% iodine in 100% ethanol for four hours, and then placed in hexamethyldisilazane for one hour and air dried overnight (Alba-Tercedor and Sánchez-Tocino, 2011; Alba-Tercedor et al., 2012; Alba-Tercedor, 2014). For the scanning process, the specimens were fixed to the tip of a small filament, as described in Alba-Tercedor and Sánchez-Tocino (2011).

The specimens were scanned using a Skyscan micro-CT attachment for the SEM. X-rays for tomography were generated with an electron beam accelerating voltage of 30 kV. To increase the beam current and, therefore, the X-ray signal, we increased the ESEM final aperture from 200 µm to 500 µm, thus getting an average gain of 4 (parameters: frame average = 3-5, depending on sample nature; pixel size = 1.2 µm; rotation step = 0.45º until completion of 180º of rotation).

The Bruker-Skyscan free software NRecon, CTAn, DataViewer and CTvox were used to reconstruct and process images. During reconstruction with NRecon, besides the normal tuning procedure, we performed x/y alignments, both an x/y iterative and an x/y alignment with a reference scan. By changing the transfer functions (obtaining consecutive overlapping symmetrical curves of the channels for the red, blue and green colors), and depleting the opacity curve within the CTvox software, it was possible to get the colors appearing in the images to point out internal structures. To correct the position of the sample we use both DataViewer, and/or CTvox: this facilitated the acquisition of totally parallel cuts of the volume renderings when using the 'Clipping Box' of Ctvox (Alba-Tercedor, 2014).

RESULTS

The study shows most of the gross internal anatomical structures described previously for the genus by TEM examination. For a detailed comparison see Figure 21 and 22. The most important result is the possibility to integrate the 3D relationships of organ systems inside the animal. Further details include the strikingly posterior position of the retracted pharynx complex, including the brain, and the presence of a large buccal tube inside the mouth cone.

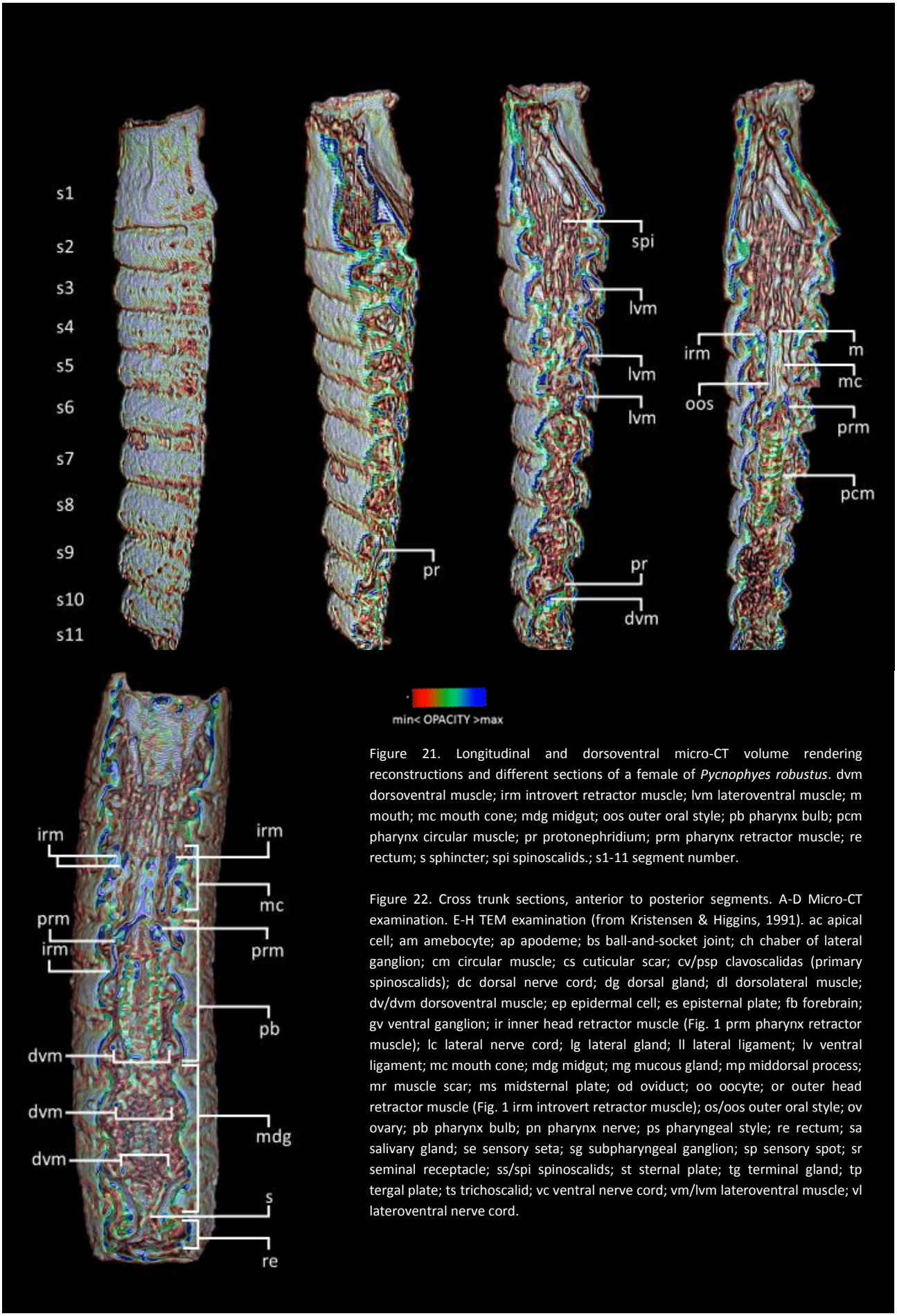
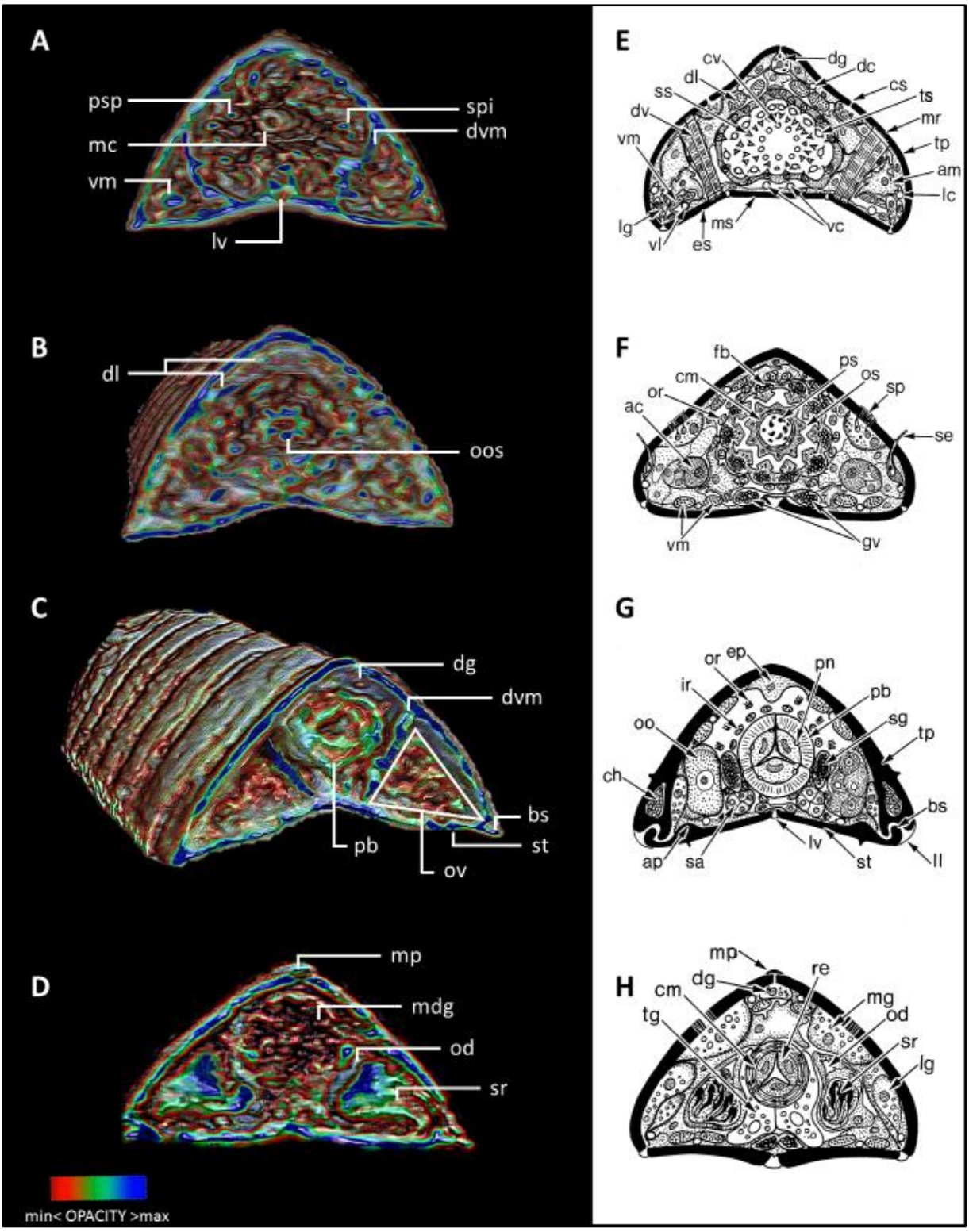


Figure 21. Longitudinal and dorsoventral micro-CT volume rendering reconstructions and different sections of a female of *Pycnophyes robustus*. dvm dorsoventral muscle; irm introvert retractor muscle; lvm lateroventral muscle; m mouth; mc mouth cone; mdg midgut; oos outer oral style; pb pharynx bulb; pcm pharynx circular muscle; pr protonephridium; prm pharynx retractor muscle; re rectum; s sphincter; spi spinoscalids.; s1-11 segment number.

Figure 22. Cross trunk sections, anterior to posterior segments. A-D Micro-CT examination. E-H TEM examination (from Kristensen & Higgins, 1991). ac apical cell; am amebocyte; ap apodeme; bs ball-and-socket joint; ch chaber of lateral ganglion; cm circular muscle; cs cuticular scar; cv/psp clavoscalidas (primary spinoscalids); dc dorsal nerve cord; dg dorsal gland; dl dorsolateral muscle; dv/dvm dorsoventral muscle; ep epidermal cell; es episternal plate; fb forebrain; gv ventral ganglion; ir inner head retractor muscle (Fig. 1 prm pharynx retractor muscle); lc lateral nerve cord; lg lateral gland; ll lateral ligament; lv ventral ligament; mc mouth cone; mdg midgut; mg mucous gland; mp middorsal process; mr muscle scar; ms midsternal plate; od oviduct; oo oocyte; or outer head retractor muscle (Fig. 1 irm introvert retractor muscle); os/oos outer oral style; ov ovary; pb pharynx bulb; pn pharynx nerve; ps pharyngeal style; re rectum; sa salivary gland; se sensory seta; sg subpharyngeal ganglion; sp sensory spot; sr seminal receptacle; ss/spi spinoscalids; st sternal plate; tg terminal gland; tp tergal plate; ts trichoscalid; vc ventral nerve cord; vm/lvm lateroventral muscle; vl lateroventral nerve cord.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I.II. DESCRIPTION OF MORPHOLOGICAL CHARACTERS OF PYCNOPHYIDAE AND NEOCENTROPHYIDAE

1) **Short LTS/TL:** lateral terminal spines/total length: measurements for the lateral terminal spines are taken from their bases to the tips. Lateral terminal spines are considered as short when the proportion is lower than 20%.

2) **Large LTS/TL:** see **"Short LTS/TL" (character 1).** Lateral terminal spines are considered as large when the proportion is over 30%.

3) **Dagger-like structure on segment 9:** (0) absent; (1) present. Sclerotized and pointed cuticular structure present on some of the Pycnophyidae species without lateral terminal spines as well as in Neocentrophyidae.

4) **TL:** Total trunk length: measured along the trunk midline, from the most anterior margin of the first segment to the most posterior margin of the eleven segments.

5) **MSW/TL:** maximal sternal width/total length: maximal sternal width was measured between the anterolateral margins of the broadest sternal plates.

INTROVERT AND MOUTH CONE

6) **Size of outer oral styles:** (0) size of outer oral styles alternating between larger and smaller; (1) all outer styles of same size. Oral styles are spinose cuticular appendages associated with the mouth cone and arranged radially into circles or rows. The outer oral styles refer to nine of these cuticular appendages, the most externally located. They are arranged as one by each sector except for sector 6 (middorsal position), which is lacking. The outer oral styles have same size in Pycnophyidae species, whereas in Neocentrophyidae and *Echinoderes* species the size alternates between larger and smaller (differences on size between the styles are much conspicuous in Neocentrophyidae species). The character was coded by direct observation for *P. oshoroensis*, *Pycnophyes* 2012a, *P. dentatus*, *P. zelinkaei*, *P. tubuliferus*, *K. yushini*, *P. aulacodes*, *P. chalgap*, *P. communis*, *P. cristatus*, *P. kielensis*, *P. lageria*, *P. smaug*, *Pycnophyes* sp. nov. 4, *K. giganteus*, *Mixtophyes abyssalis*, *Paracentrophyes anurus*, *Paracentrophyes quadridentatus*, *E. sensibilis and E. rex*. Data for *P. parasanjuanensis*, *K. phyllotropis*, *N. intermedius*, *N. satyai* and *Paracentrophyes praedictus* have been taken from the literature. The presence of outer oral styles of same size is a well settled diagnostic character in Pycnophyidae; hence this character state is assumed for the remaining species of the family.

7) **Articulated outer oral styles:** (0) absent; (1) present. The outer oral styles consist of two articulating units or a single unit. In *Echinoderes* species, the nine outer oral styles are robust and articulated, whereas articulated styles are absent in Pycnophyidae. However, Neocentrophyidae species present both articulated and non-articulated styles: five large and well-developed styles consisting of two articulating units, alternating with four smaller, non-articulated ones. The character was coded by direct observation for *P. oshoroensis*, *Pycnophyes* 2012a, *P. dentatus*, *P. zelinkaei*, *P. tubuliferus*, *K. yushini*, *P. aulacodes*, *P. chalgap*, *P. communis*, *P. cristatus*, *P. kielensis*, *P. lageria*, *P. smaug*, *Pycnophyes* sp. nov. 4, *K. giganteus*, *Mixtophyes abyssalis*, *Paracentrophyes anurus*, *Paracentrophyes quadridentatus*, *E. sensibilis* and *E. rex*. Data for *P. parasanjuanensis*, *K. phyllotropis*, *N. intermedius*, *N. satyai* and *Paracentrophyes praedictus* were taken from the literature. The presence of articulated outer oral styles had never been reported for Pycnophyidae species (which always bear unarticulated styles), hence we presume this trait is absent in all the species of the family.

8) **Non-articulated outer oral styles:** (0) absent; (1) present. See "**Articulated outer oral styles" (character 7):** The character was coded by direct observation and published data for the same species as in character 7. The presence of non-articulated outer oral styles only is a diagnostic character for Pycnophyidae, so we presume this character is present in all the species of the family.

9) **Number of dorsal trichoscalids:** (0) 7 trichoscalids; (1) 4 trichoscalids. Trichoscalids are modified sensorial head appendages with feather shaped. They are located in the posteriormost introvert ring. The observation of the character was possible in all the species for which their vouchers were studied (*P. rugosus*, *P. ponticus*, *P. flaveolatus*, *P. tubuliferus*, *P. oshoroensis*, *Pycnophyes* 2012a, *Pycnophyes* 2012b, *P. dentatus*, *P. robustus*, *P. zelinkaei*, *P. tubuliferus*, *P. yushini*, *Paracentrophyes anurus*, *Paracentrophyes quadridentatus*, *E. sensibilis*, *E. rex*) as well as in all the following species*: P. almansae*, *P. aulacodes*, *P. carinatus*, *P. chalgap*, *P. communis*, *P. cristatus*, *P. dolichurus*, *P. farinelli*, *P. frequens*, *P. kielensis*, *P. lageria*, *P. norenburgi*, *P. nubilis*, *P. pardosi*, *P. smaug*, *P. sp4*, *P. sp5*, *P. argentinensis*, *P. beaufortensis*, *P. ecphantor*, *P. borealis*, *P. corrugatus*, *P. cryopygus*, *P. egyptensis*, *P. emarginatus*, *P. greenlandicus*, *P. iniorhaptus*, *P. longicornis*, *P. neuhausi*, *M. abyssalis*, *N. intermedius*, *N. satyai*. Data for *Paracentrophyes praedictus and K. phyllotropis* are available in the literature. Original description of *P. australensis* and *K. ilyocryptus* give information about the total number of trichoscalids only (14), but there is not data on its distribution. Information given for *P. newzealandiensis* and *P. spitsbergensis* might be mistaken, 13 and 15 trichoscalids respectively. Observation of this character under LM, as is the case, may be misleading. For these two species and for the remaining ones, we assume the presence of 7 dorsal trichoscalids for all Pycnophyidae species due to it is a diagnostic character for the family.

10) **Number of ventral trichoscalids:** (0) 7 trichoscalids; (1) 2 trichoscalids. See "**Number of dorsal trichoscalids" (character 9)**. The observation of the character was possible for the same species as in character 9. Data on *Paracentrophyes praedictus*, *K. phyllotropis*, *P. australensis*, *K. ilyocryptus*, *P. newzealandiensis*, *P. spitsbergensis* same as in character 9. We assume the presence of 7 ventral trichoscalids for all remaining species of Pycnophyidae due to it is a diagnostic trait for the family.

11) **Trichoscalid plates:** (0) absent; (1) present. Cuticular plates that bear trichoscalids and articulated to a neck placid. The character is absent in all species of Pycnophyidae and Neocentrophyidae, whereas is present in all *Echinoderes*.

NECK

12) **Number dorsal placids:** (0) 4; (1) 7 or more. The character refers to the number of placids, or introvert closing plates, that are located in the dorsal side. Pycnophyidae and Neocentrophyidae species always have four dorsal placids (diagnostic character), whereas its number is higher in *Echinoderes* species (7 or 9 if the lateral ones are considered in the dorsal side). No available data for P. longicornis, *P. arctous*, *P. calmani*, *P. maximus* and *K. paraneapolitanus*, so the character was coded as missing data. The presence of four placids was confirmed by type material for *K. stenophygus*. Data for *P. odhneri* and *K. anomalus* are considered as mistaken and coded as missing data due to refer to the presence of 3 and 2 dorsal placids respectively.

13) **Number ventral placids:** (0) 7 or more; (1) 4; (2) 3; (3) 2. The character refers to the number of placids, or introvert closing plates, that are located in the ventral side. The number of ventral placids can either be two or four in Pycnophyidae species, whereas its number is always fixed in Neocentrophyidae and *Echinoderes* species, 3 and 7 respectively (the number of ventral placids in *Echinoderes* would be 9 if the lateral ones are considered in the ventral side). The character was coded by direct observation for all the vouchers as well as *P. beaufortensis*, *P. ecphantor*, *P. egyptensis*, *P. emarginatus*, *P. iniorhaptus*. Data for *P. canadensis* was taken from the original description (picture number 12), as well as for *K. cataphractus* (LM pictures in Adrianov and Malakhov). Even though the original description of *K. phyllotropis* reports the presence of either three or four ventral placids, we verified that there are two ventral placids actually. No available and therefore coded as missing data for *P. longicornis*, *P. arctous*, *P. maximus*, *P. parasanjuanensis*, *K. paraneapolitanus* and *Pycnophyes* sp. nov. 5. We preferred to code this character as missing data for *P. frequens* (not noticeable in the studied species), *P. odhneri*, *K. anomalus* (the original description in both species refers to three dorsal placids, and four and two ventral placids respectively, but it might be mistaken also in the ventral side), *K. apotomus* (four ventral placids according to the original description, but under direct observation we are not sure about whether there are two or four

placids actually), *K. fimbriatus*, *K. stenophygus* (two ventral placids according to the original description, but we are not sure about whether there are two or four actually) and *K. spinosus* (old literature where the author describes the presence of three ventral placids, never reported again).

OVERALL TRUNK

14) **Trunk shape in cross section:** (0) triangular; (1) rounded. Pycnophyidae and Neocentrophyidae kinorhynchs have a trunk conspicuously triangular in cross-section, whereas in cyclorhagids the trunk is generally rounded, heart-shaped or elliptical.

15) **Composition of segment 1**: (0) ring; (1) two plates, one dorsal and one ventral; (2) two plates, ventral one with initial divisions; (3) one dorsal and three ventral plates. Segment 1 consists of one closed ring in the cyclorhagid outgroups taxa. Among species of Pycnophyidae the first trunk segment consists of one tergal and three sternal plates, whereas species of Neocentrophyidae have one tergal and one sternal plate, with the ventral one being just partially divided anteriorly in *Paracentrophyes* or undivided in *Neocentrophyes* and *Mixtophyes*.

16) **Composition of segment 2:** (0) ring; (1) one tergal and two sternal plates. Segment 2 always consists of one closed ring in *Echinoderes* species, whereas it is always divided into one dorsal (tergal) and two ventral (sternal) plates in Pycnophyidae and Neocentrophyidae.

17) **Composition of segment 11:** (0) one tergal and one sternal plates; (1) one tergal and two sternal plates. The sternal region of terminal trunk segment is divided into two plates in all *Echinoderes* and Pycnophyidae species, whereas is formed by a single, undivided plate in Neocentrophyidae.

18) **Position of penile spines:** (0) between segment 10 and 11; (1) one on segment 10 and one on segment 11; (2) segment 10. Penile spines are flexible appendages present bilaterally at the posterior segments in males of most kinorhynch species. The penile spines are located between the two last segments in *Echinoderes* and Pycnophyidae species, whereas they may vary among Neocentrophyidae species: one pair on segment 10 and one pair on segment 11 in *Paracentrophyes* and *Neocentrophyes*, or has just a single pair located on segment 10 in Mixtophyes. The character was not available to check in those species coded by female vouchers (*P. ponticus*, *P. dentatus*, *P. robustus* and *P. tubuliferus*), although it was observed in males of the additional material collected at the same locality as the voucher. The character was coded as inapplicable for *P. nubilis*, *P. argentinensis*, *K. rabaulensis* and *N. satyai* because of only females are known.

19) **Presence of middorsal spine:** (0) absent; (1) present. Middorsal spines are unpaired cuticular appendages of the trunk with an articulated proximal basis and a longer, rigid distal part, ending in a pointed and closed tip. They are present on some or every

segments 4 to 8 in *Echinoderes*, and restricted to segments 10 and 11 (the former one just in males) in Neocentrophyidae. Contrarily, all Pycnophyidae species lack this character.

20) **Middorsal spine of segment 11:** (0) absent; (1) present. See character "**Presence of middorsal spine"** (**character 19)**. The character is present in all Neocentrophyidae species and absent in *Echinoderes*. The character was coded as inapplicable for Pycnophyidae.

21) **Pachycyclus:** (0) present; (1) absent. Inner thickening of the anterior edge of the segment. The character is present in all Pycnophyidae species as well as in *Echinoderes*, whereas it is absent in Neocentrophyidae.

22) **Lateral terminal structures of segment 11:** (0) spines; (1) protuberances. Most kinorhynch species bear a pair of large lateral terminal spines on the last trunk segment. *Kinorhynchus* and *Neocentrophyes* species bear a pair of rounded, bulbous, articulated protuberances instead. *Pycnophyes*, *Paracentrophyes* and *Mixtophyes* species have lateral terminal spines. The character was coded as missing data in *K. yushini*, *K. deirophorus*, *K. cataphractus*, *K. spinosus* because the presence of bulbous protrusion could not be confirmed.

23) **Ventral tubes on segment 2 in males:** (0) absent; (1) present. Named 'adhesive tubule' in older literature, but this assumption should be avoided until its actual function (secretory/adhesive or sensory) is confirmed. Sexual dimorphism in species of Pycnophyidae is expressed by the presence of penile spines on the last trunk segment and usually by one pair of large ventral tubes on segment 2 (present in all males of *Kinorhynchus* and, supposedly, lacking in only eight males out of 57 species of *Pycnophyes* described so far). These tubes are elongated cuticular appendages present on the sternal plates in males, with an articulated proximal basis and a longer, flexible and tubular distal part, ending in a terminal opening. The lack of this character was directly observed in the voucher of *P. rugosus*, but not in the voucher of *P. ponticus* (since it is a female) although it was observed in males of the additional material collected at the same locality as the voucher. Also it was observed in the type material of *P. dolichurus*, *P. farinelli*, *P. ecphantor*, *P. egyptensis*, *P. longicornis* and in two new species, *Pycnophyes* sp. nov. 4, *Pycnophyes* sp. nov. 5. Information about the absence of the character for *P. chilensis* was taken from the literature. The character was coded as inapplicable for *P. nubilis*, *P. argentinensis*, *K. rabaulensis* and *N. satyai* because only females are known.

24) **Midventral midsternal projection:** (0) absent; (1) present. Pointed, non-articulated projection of the posterior ventral margin of the first trunk segment that extends posteriorly from the midventral position. Although this character may be difficult to check in some specimens, it is present in *P. aulacodes*, *P. communis*, *P. frequens*, *P.*

norenburgi, *P. smaug*, *P. barentsi* (see Fig. 5.68 C, 5.69A in Adrianov and Malakhov 1999a), *P. schornikovi* (see Fig. 5.109 A in Adrianov and Malakhov 1999a) and *P. greenlandicus* (original description does not report this character but it seems to be present or suggested in Fig. 150, Higgins and Kristensen, 1988; and it was also checked by direct observation of additional material). The character is absent in all vouchers and in the remaining species coded by direct observation, except for *P. chalgap*, where it is coded as missing (with a special structure at the posterior margin of the midsternal plate, rounded and large, extending beyond the anterior half of the following segment). No available data for *P. calmani* and *P. maximus*, therefore the character was coded as missing data for both species.

25) **Kind of sensory spot:** (0) flosculi-like; (1) several flosculi; (2) typical type 1 or 2**.** Sensory spots are cuticular specializations for the reception of sensory stimuli. For most kinorhynchs sensory spots consist of a little round to oval area with many micropapillae (up to 100), with several pores and cilia (type 1 and type 2). Three species of Pycnophyidae possess a special kind of sensory spots type 1, commonly named flosculi or *Nanaloricus*-flosculi (N-flosculi), with a reduced number (8-10) of cuticular papillae, arranging in a single circle and surrounding a central pore: *P. lageria*, *K. yushini* and *K. ilyocryptus* (not described for the latter species, but it is shown in Fig. 5.127, Adrianov and Malakhov 1999a). This kind of spot is also present in all species of Neocentrophyidae, but consisting of several joined flosculi. The kind of sensory spots is easily confirmed by SEM observations, whereas confirmations by LM are much more difficult. Hence it was not possible to confirm the character for the species checked or described by LM only: *Pycnophyes* 2012a, *P. farinellii*, *P. nubilis*, *Pycnophyes* sp. nov. 4, *Pycnophyes* sp. nov. 5, *P. beaufortensis*, *P. emarginatus*. For the species coded by the literature only, in order to avoid too much missing data, we coded the character as typical type 1 or 2 when it is drawing as a circle with two points inside (it is supposed representing the sensory spots area -circle- and the number of pores); in other way, it was coded as missing data. For those species described by SEM, data were taken from the literature.

26) **Protonephridial opening:** (0) cribate; (1) multitubular. The protonephridia open to the outside by two different systems of bilateral cuticular perforations located on segment 9: cribate plate (sieve plate) in *Echinoderes* species or tubular set in Pycnophyidae and Neocentrophyidae. This character was impossible to check in the vouchers but it was observed in additional material of the same species by SEM or the character was coded taken data from the literature (except for *Pycnophyes* 2012a, *Pycnophyes* 2012b, *P. tubuliferus*). The character was coded by direct observation in *P. almansae*, *P. aulacodes*, *P. carinatus*, *P. chalgap*, *P. communis*, *P. cristatus*, *P. dolichurus*, *P. frequens*, *P. lageria*, *P. norenburgi*, *P. pardosi*, *P. smaug*, *P. australensis* (see Fig. 5F in Lemburg 2002), *P. abyssorum* (Adrianov and Maiorova 2014), *P. ilyocryptus* (Adrianov and Malakov 1999a), *M. abyssalis* and *Paracentrophyes praedictus* (Fig. 96, Higgins 1983). Due to the

observation of the character may be difficult by LM, we assumed the presence of multitubular protonephridial openings for all Pycnophyidae species studied by ourselves or described with LM only.

27) **Ball (or peg) and socket joints:** (0) 2-10; (1) 2-9; (2) 2-8; (3) 2-7; (4) 2-5; (5) 2-3; (6) absent. The joint area between the dorsal and ventral pachycycli is present at the anterior tergal-sternal junction on some segments, which is defined as an inner thickening of the anterior edge of each segment plate, growing transversally to the interior to form an inner cuticular ring, and serving as the attachment point for trunk muscles. Ball and socket joints are well-developed in *Pycnophyidae* species, but its presence on the segments has a high variability between the species. All Neocentrophyidae species lack this structure. The character was coded as inapplicable for *Echinoderes* species.

28) **Dorsal cuticular scars:** (0) scattered dot-shaped; (1) rounded-oval; (2) dotted line; (3) groove-shaped. Cuticular scars are surface openings of internal glands. They appear arranged segmentally in both Cyclorhagida and Allomalorhagida, usually conspicuous but only detectable with LM, and their shape are useful as taxonomic tool. The character could not be observed and coded as missing data in *P.* 2012a, *K. yushini*, *P. cryopygus*, *P. chukchiensis*, *K giganteus* (SEM observation only), as well as in the *Echinoderes* species. We also coded the character as missing data for *P. australensis* and *P. norenburgi* because the shape showed an intermediate stage between groove-shaped and rounded-oval. Even though the observation of the character is very easy with LM, much of the old literature does not include any data about them, or data are confusing (as in *P. newguiniensis*) and the character was coded as missing data. The character was coded as rounded-oval for *K. belizensis* and *K. mainensis* by direct observation of type material. The original description *of K. distentus* reports that the cuticular scars are crescentic, however, they are rounded-oval according to the attached pictures in the description.

29) **Ventral cuticular scars:** (0) scattered dot-shaped; (1) rounded-oval; (2) dotted line; (3) groove-shaped, crescentic. See Character 28 "**Dorsal cuticular scars**". As well as for the dorsal cuticular scars much of the old literature does not include any data about them, or the data are confusing therefore data for these species were coded as missing data. The character could not be observed and coded as missing data for *K. yushini*, *P. kielensis* and the *Echinoderes* species. For *P. australensis*, *P. ecphantor*, *P. corrugatus* the character was coded as missing data because the intermediate shape between groove and rounded-oval. This character is described as rounded-oval for *K. erismatus* and *K. langi*, but they are groove-shaped actually. The character was coded as groove-shaped for *P. neuhausi* and rounded-oval for *K. mainensis* by direct observation of type material.

30) **Midsternal anterior ornamentation:** (0) absent; (1) present. Cuticular ornamentation located at the anteriormost region of the midsternal plate (segment 1, central plate of the ventral side). The character was coded as missing data for *P. beaufortensis*, *P.*

ecphantor, *P. iniorhaptus*, *P. calmani*, *P. canadensis*, *P. galtsovae*, *P. maximus*, *P. odhneri*, *K. paraneapolitanus*, *K. spinosus* and *K. trisetosus* because there is not information in the description or the character could not be observed in the type material. The character was checked in additional material of *P. kielensis* and coded as absent as well as in the type material of *K. stenophygus*, coded as present. According to the literature, *K. phyllotropis* has a midsternal anterior ornamentation as circles or dots in some specimens only, therefore we coded the character as inapplicable.

TRUNK APPENDAGES

Middorsal structure specialization: Any of the cuticular structures present at the posterior margin of the tergal plates. The middorsal structures present three different shapes. Middorsal elevations are cuticular structures poorly developed, not protruding beyond the segment margin, usually bearing hairs along the middorsal line. Middorsal processes are protruding structures that surpass beyond the posterior margin of the segment. Usually with hairs along the middorsal line. These may be just a pointed protrusion of the posterior segment margin or have a keel-shaped with a rigid terminal end. Middorsal spinose processes are non-articulated pointed projection of the posterior edge of the tergal plate. These have a conspicuous keel-shape, with an elongate base and a flexible terminal end, beginning at the anterior third of the segment and surpassing half of the following segment. Middorsal spines are unpaired cuticular appendage of the trunk with an articulated proximal basis and a longer, rigid distal part, ending in a pointed, closed tip. They are present on some or every segments 4 to 8 in *Echinoderes*, whereas such structures are absent in all species of Pycnophyidae and Neocentrophyidae (the latter family with middorsal spines on segment 11 in both sexes and on segment 10 in males only). Several authors do not discriminate between middorsal elevations and middorsal processes, and they just report the presence of "middorsal processes (or middorsal spinose process) that surpass or not surpass the posterior segment margin" or "pointed/rounded" middorsal processes or "weakly developed lanceolate spine". Therefore, for the species coded following the information given in the literature only (text and drawing only available, not pictures to check the characters), we coded as middorsal elevations all these middorsal processes that not surpass the posterior margin of the segment, and as middorsal processes all those that actually surpass the posterior margin. As the same way, we coded as middorsal elevation all these "rounded middorsal process or weakly developed lanceolate spine". Even though the redescription of *K. yushini* does not report the presence of middorsal elevation on segment 1, it was present in the voucher.

31) **Kind of middorsal structure specialization on segment 1:** (0) middorsal elevation; (1) middorsal process; (2) middorsal spinose process; (3) smooth margin. See information of **"Middorsal structure specializations"**. *Pycnophyes barentsi* has a middorsal process on segment 1, according to Fig. 5.68A in Adrianov and Malakhov 1999a. The presence of middorsal elevations is described for *K. erismatus*, but it is not illustrated. Fortunately,

the presence of middorsal elevations could by confirmed by direct observation of the type material. As for *K. anomalus*, the authors describe the presence of middorsal spinose processes extending beyond posterior margins of segments 1-9, but then these structures are drawing as non-surpassing the posterior margin; we decided to follow the information given in the main text. Data on this character is unavailable in the literature for *K. trisetosus*, but the presence of middorsal elevations was confirmed by type material. *Kinorhynchus ilyocryptus* and *K. phyllotropis* have middorsal elevation on segment 1 according to Adrianov and Malakhov 1999a (Fig. 5.126A) and Brown and Higgins 1983 (Fig. 3A) respectively. *Kinorhynchus stenophygus* has a middorsal process on segment 1 according to Higgins 1983 (Fig. 229) and this was confirmed by the type material. Adrianov described the presence of middorsal structures short, pointed, slightly protruding beyond posterior tergal margin in *K. rabaulensis*, but then these appear surpassing the posterior segment margin just a bit (Adrianov and Malakhov 1999a), therefore the character was coded as middorsal process. Data was unavailable and coded as missing data for *P. calmani*, *P. maximus*, *P. newguiniensis*. Due to some contradictions between the text and the literature illustrations, or when no images were available to check which kind of structure is present, we decided to code the character as missing data, such as *P. galtsovae*, *P. newzealandiensis*, *P. odhneri* and *K. spinosus. Pycnophyes iniorhaptus* was coded as inapplicable since the kind of middorsal structure seems to be related with the sexes. The character could not be confirmed in the type material of *P. longicornis* so data was taken from the literature. The character was coded as missing data for *K. giganteus* since Zelinka illustrated its presence in some drawings but not in all. The literature leaves no available data for *P. calmani*, *P. maximus*, *P. newguiniensis*, so they were coded as missing data. The drawing in the description of *P. chukchiensis* shows a tiny structure in middorsal position, hence we preferred to code the character as missing data.

32) **Kind of the middorsal structure specialization on segment 2:** (0) middorsal elevation; (1) middorsal process; (2) middorsal spinose process; (3) smooth margin. See information of **"Middorsal structure specializations"** and character 31 **"Middorsal structure specialization on segment 1"**. *Kinorhynchus yushini* is described with middorsal elevations on segments 1-3, and middorsal processes on segments 4-9. However, a middorsal process is visible on segments 3-4 also in the voucher. The literature refers the absence of a middorsal structure on segment 2 for *K. belizensis*, but the presence of middorsal process was confirmed by the type material. The literature refers the presence of middorsal processes in *K. stenophygus* but the presence of middorsal elevation was confirmed by the type material. *Kinorhynchus stenophygus* has a middorsal process according to the drawing but it is actually a middorsal elevation (Higgins 1983 Fig. 229) and its presence was confirmed by the type material. The original description of *P. belizensis* reports the absence of the character on this segment, but the presence of a middorsal elevation was confirmed in type specimens. Data on this character is

unavailable in the literature for *K. trisetosus*, but the presence of middorsal elevations was confirmed in type material. Data are unavailable and coded as missing data for *P. calmani* and *P. maximus*. Due to some contradictions between the text and the literature illustrations, and when no images are available to check which kind of structure is present, we decided to code the character as missing data, such as *P. odhneri*, *K. paraneapolitanus* and *K. spinosus*. As for *K. anomalus*, the authors described the presence of "middorsal spinose process extending beyond posterior margins on segments 1-9", but then these structures are drawn as non-surpassing the posterior margin; we decided to follow the information given in the main text. In *K. cataphractus*, the middorsal structure does not seem to surpass the posterior margin of the segment, and, moreover, it looks the same in the images in Adrianov and Malakhov (1999a) (Fig. 5.122B-C). *Kinorhynchus ilyocryptus* has a middorsal elevation on segment 1 according to Adrianov and Malakhov 1999a (Fig. 5.126A). Adrianov described the presence of short middorsal structures, pointed, slightly protruding beyond posterior tergal margin in *K. rabaulensis*, but then these appear surpassing the posterior segment margin just a bit (Adrianov and Malakhov 1999a), therefore the character was coded as middorsal process. Adrianov described the presence of obtuse or underdeveloped middorsal structures (elevations) on segments 1-6 in *P. arctous*; however we preferred to code the character as missing data on these segments because the presence of middorsal elevations together with highly developed keel-shaped middorsal processes on the remaining segments had never been reported.

33) **Kind of middorsal structure specialization on segment 3:** (0) middorsal elevation; (1) middorsal process; (2) middorsal spinose process; (3) smooth margin. See information of **"Middorsal structure specializations"** and characters 31 and 32 **"Shape of the middorsal structure specialization segment 1 and 2"**. Same coding as in character 32 for *K. yushini*, *P. arctous*, *P. calmani*, *P. maximus*, *P. odhneri*, *K. anomalus*, *K. cataphractus*, *K. ilyocryptus*, *K. spinosus*, *K. stenophygus*, *K. rabaulensis*. The description of *K. fimbriatus* gives information about the presence of middorsal elevations on segments 1 and 2 only, but its presence was observed until segment 6 in the type material.

34) **Kind of middorsal structure specialization on segment 4:** (0) middorsal elevation; (1) middorsal process; (2) middorsal spinose process; (3) spine; (4) smooth margin. See information of **"Middorsal structure specializations"** and characters 31 and 32 "**Shape of the middorsal structure specialization segment 1 and 2"**. The character coding for *P. fimbriatus*, *P. arctous*, *P. calmani*, *P. maximus*, *P. odhneri*, *K. anomalus*, *K. cataphractus*, *K. ilyocryptus*, *K. spinosus*, *K. stenophygus*, *K. rabaulensis* follows the reasons given in character 33 **"Middorsal structure specialization on segment 3"**.

35) **Kind of middorsal structure specialization on segment 5:** (0) middorsal elevation; (1) middorsal process; (2) middorsal spinose process; (3) spine; (4) smooth margin. See information of **"Middorsal structure specializations"** and characters 31 and 32 "**Shape of**

the middorsal structure specialization segment 1 and 2". The character encoding for *P. fimbriatus*, *P. arctous*, *P. calmani*, *P. maximus*, *P. odhneri*, *K. anomalus*, *K. ilyocryptus*, *K. spinosus*, *K. stenophygus*, *K. rabaulensis* follows the reasons given in character 33 "**Middorsal structure specialization on segment 3"**. *Kinorhynchus cataphractus* was coded as missing data because it was impossible to determinate properly if the middorsal structure surpasses or not the posterior margin of the segment (see Fig.5.122B-C in Adrianov and Malakhov 1999a).

36) **Kind of middorsal structure specialization on segment 6:** (0) middorsal elevation; (1) middorsal process; (2) middorsal spinose process; (3) spine; (4) smooth margin. See information of **"Middorsal structure specializations"** and characters 31 and 32 **"Shape of the middorsal structure specialization segment 1 and 2"**. The character coding for *P. fimbriatus*, *P. arctous*, *P. calmani*, *P. maximus*, *P. odhneri*, *K. anomalus*, *K. cataphractus*, *K. ilyocryptus*, *K. spinosus*, *K. stenophygus*, *K. rabaulensis* follows the reasons given in the character 33 **"Middorsal structure specialization on segment 3"**. Even though the original description of *K. langi* does not include the presence of middorsal elevation on segment 6, it was confirmed by the type material (middorsal elevation on segments 1-6). *Kinorhynchus cataphractus* was coded as missing data because it was impossible to determinate properly whether or not the middorsal structure surpasses the posterior margin of the segment (see Fig.5.122B-C in Adrianov and Malakhov 1999a).

37) **Kind of middorsal structure specialization on segment 7:** (0) middorsal elevation; (1) middorsal process; (2) middorsal spinose process; (3) spine; (4) smooth margin. See information of **"Middorsal structure specializations"** and characters 31 and 32 **"Shape of the middorsal structure specialization segment 1 and 2"**. The character coding for *P. calmani*, *P. maximus*, *K. anomalus*, *K. ilyocryptus*, *K. spinosus*, *K. stenophygus*, *K. rabaulensis* follows the reasons given in the character 33 **"Middorsal structure specialization on segment 3"**. *Pycnophyes galtsovae* has middorsal elevations in females, but any data is given for males (Adrianov and Malakhov 1999a), hence the character was coded as inapplicable. There are not available images for *P. odhneri*, *K. cataphractus*, *K. spinosus*, but in the illustrations the structures seem to clearly surpass the posterior margin, hence we coded the character as middorsal process. The illustration of *P. sanjuanensis* shows that the middorsal structure could slightly surpass the posterior margin of the segment, but in case of doubt we preferred to code the character as missing data. In *K. erismatus* the structure was described as middorsal elevation on segments 7-9, but the observation of type material showed that the structures are middorsal processes actually. In *K. fimbriatus* a slight structure on segments 7 and 8 is showed in the illustration but its absence was confirmed for both segments in the type material. In *K. mainensis* the study of the type material confirmed the absence of middorsal structure specializations on segments 7-10.

38) **Kind of middorsal structure specialization on segment 8:** (0) middorsal elevation; (1) middorsal process; (2) middorsal spinose process; (3) spine; (4) smooth margin. See information of **"Middorsal structure specializations"** and characters 31 and 32 **"Shape of the middorsal structure specialization segment 1 and 2"**. The character coding for *K. anomalus*, *K. ilyocryptus*, *K. spinosus*, *K. stenophygus*, *K. rabaulensis*, *P. galtsovae, P. odhneri*, *K. cataphractus*, *K. spinosus*, *P. sanjuanensis*, *K. erismatus*, *K. fimbriatus*, *K. mainensis* follows the reasons for character **"Middorsal structure specialization on segment 7"**. According to the description of *P. faveolus* there is not middorsal structure on any segment, but the illustration shows a poorly developed structure on this segment, hence to avoid mistakes we preferred to code the character as missing data. The character was coded as missing data for *K. belizensis* because we are not sure about what kind of middorsal structure is present. The trait is coded as middorsal elevation for *K. deirophorus* and *K. distentus* because its presence was confirmed in the type material, even though it is not described in the original descriptions. The character state for *K. stenophygus* could not be confirmed and coded as missing data. Same coding as in character 33 for *P. calmani*, *P. maximus.*

39) **Kind of middorsal structure specialization on segment 9:** (0) middorsal elevation; (1) middorsal process; (2) middorsal spinose process; (3) smooth margin. See information of **"Middorsal structure specializations"** and characters 31 and 32 **"Shape of the middorsal structure specialization segment 1 and 2".** Data for *K. belizensis* and *K. trisetosus* could not be confirmed by the type material and therefore coded as missing data. Main text of original description of *P. faveolus* does not report information about middorsal structure on this segment but the drawing shows that a structure is present, since we preferred to code the character as missing data. The character coding for the remaining species follows the reasons given for character 37 **"Middorsal structure specialization on segment 7"**, except for *K. yushini* (males with middorsal process and females with middorsal structure, hence the character was coded as inapplicable), *K. ilyocryptus* (it was impossible to confirm the presence of a middorsal elevation in Fig. 5.126, in Adrianov and Malakhov 1999a, hence we preferred to code it as missing data).

40) **Middorsal structure specialization on segment 10 (at least present in one sex):** (0) smooth margin; (1) middorsal elevation; (2) middorsal process; (3) spine. See information of **"Middorsal structure specializations"**.

41) **Middorsal structure specialization on segment 10 conspicuously extended over the segment 11**: (0) absent; (1) present. See information of **"Middorsal structure specializations"**. Some allomalorhagid species show the middorsal process of segment 10 conspicuously extended beyond the segment edge, as a keel-like structure with a rigid pointed tip. This structure is absent in all Neocentrophyidae and *Echinoderes* species as well as in mostly allomalorhagid species, except for *P. cristatus*, *P. nubilis*, *P. arctous*, *P. chukchiensis*, *P. furugelmi* and *P. abyssorum*.

Setae: the term refers to elongated cuticular appendages of the trunk, with a proximal basis articulated to the segment plate and a longer, flexible and tubular distal part, flanked by two lateral, flat rims and ending in a terminal opening.

Dorsal series: Setae in paradorsal (PD) and laterodorsal (LD) position were coded. Paradorsal setae are those located immediately adjacent to the middorsal position of the segment. As far as we know from our personal observations, setae do not appear in middorsal positions, but they present always a slight lateral shift, appearing located in paradorsal position. Laterodorsal setae are those located bilaterally on the ventralmost 50% of the tergal area, between the paradorsal position and the widest point of the trunk. Both positions can only be observed from the dorsal side of a dorsoventrally mounted specimen. Even though the descriptions of *P. arctous*, *P. calmani*, *P. chilensis* and *P. maximus* do not include information about presence of any setae along the whole trunk we preferred to code it as missing data rather than "absent" because setae are sometimes difficult to see using LM only when the specimen is not in well conditions. As for *P. galsovae*, there is no available data about the presence of paradorsal or middorsal setae, but the strange setae distribution in the remaining position and the absence of any lateral setae, which leads us to assume that the information of the description may be incomplete or mistaken. The presence of setae is described at the lateral side only for *K. spinosus* and for the ventral and lateral sides only in *P. odhneri*. For all these cases, paradorsal and laterodorsal setae were coded as missing data instead of absent.

42) **MD/PD setae on segment 1:** (0) absent; (1) present. See information of **"Dorsal series"**. Setae in subdorsal position are presents in *P. canadensis*, *P. carinatus*, *P. abyssorum*, *K. anomalus*, *K. paraneapolitanus*, *K. phyllotropis*, *K. stenophygus*, but not in paradorsal position. The original description of *P. ilyocryptus* does not give information about this character, but its absence was checked in Adrianov and Malakhov a (Fig. 5. 126 A). The character for *P. arctous*, *P. calmani*, *P. chilensis*, *P. galtsovae*, *P. maximus*, *P. odhneri*, *K. spinosus* was coded as missing data according to the reasons given in **"Dorsal series"**.

43) **PD setae on segment 2:** (0) absent; (1) present. See information of **"Dorsal series"**.

P. arctous, *P. calmani*, *P. chilensis*, *P. galtsovae*, *P. maximus*, *P. odhneri*, *K. spinosus* were coded as on segment 1 following the same reasons. The voucher of *P. oshoroensis* has a mark in this position that could be a setae perforation site. The character has a high intraspecific variability since it is absent in the holotype out of 11 specimens, and the setae are paired in one paratype, whereas a single setae is present only in 9 specimens. Given this intraspecific variability as well as the impossibility of a proper identification of the character state, we preferred to code the character as inapplicable. Even though the original description of *P. argentinensis* reports the presence of some protuberances in several segments that could be setae perforation sites, we verified that these are not setae actually

(absent on segments 1-10). Same for *K. langi*, for which the character is described as suggested on this segment (as well on segments 4 and 6) but its absence on segment 2 was confirmed in the type material. As for *P. corrugatus* and *P. iniorhaptus*, the presence of cuticular marks that could be the setae perforation site was observed in the type material, but we could not see the setae. Same for *K. stenophygus*, with marks on segments 2, 4, 6, 8 and 9. Therefore, we preferred to code the character as missing data for *P. corrugatus* on segments 2- 9, for *P. iniorhaptus* on segments 2-8 and for *K. stenophygus* on segments 2, 4 and 9 (confirmed on segments 6, 8). Even though the illustrations of *P. newzealandiensis* show a protuberance in paradorsal position on this segment, in the main text the presence of paradorsal setae is specified (same problem for segments 4, 5, 7 and 8). For several species, such as *P. sanjuanensis* and *K. cataphractus* (see Fig. 5.122 in Adrianov and Malakhov 1999a) the presence of protuberances is described, but it was impossible to know whether these are actually setae and therefore the character was coded as missing data (protuberances are described for both species on segments 2-9). In other species, such as *K. deirophorus* (see Fig. 234 and 235 in Higgins 1983), *K. distentus* (Fig. 250 Higgins 1983), *K. erismatus* (see Fig. 272 and 273 in Higgins 1983) and *K. rabaulensis* the authors drew circles or similar structures in the illustrations that could be setae perforation sites. Observations of the type material of *K. deirophorus* (segments 2, 4, 6, 8-9), *K. distentus* (segments 2, 4, 6, 8) and *K. erismatus* corroborated that these structures are actually setae. The presence of paradorsal setae in *K. rabaulensis* could not be confirmed by the type material, so the character was coded as missing data. The original description of *K. phyllotropis* does not report the presence of paradorsal setae on any segment, but its presence on segments 2, 5, 6, 8 was confirmed by the observation of the type material (doubts on segments 4 and 9 lead us to code the character on these segments as missing data). Paradorsal setae are present in females but not in males of *P. longicornis*, so the character was coded as inapplicable. Similar in *K. anomalus*, for which the character is present at least in females but there is not data for males. *Kinorhynchus belizensis* is described with paradorsal setae on this segment but only in some specimens; however we observed paradorsal setae in all specimens of the type material, so it was finally coded as present. *P. neuhausi* was described without any paradorsal setae, but the presence of paradorsal setae on segments 2-9 was observed in the type material. In the illustrations of *K. giganteus*, a circle is drawing in middorsal/paradorsal position on segment 2 (moreover, SEM pictures taken of additional material showed a possible seta on segments 4, 6, 8). Therefore, we coded the character as missing data for this segment. Higgins described the presence of protuberances in *K. ilyocryptus*, but latter Boykin described the presence of the paradorsal setae on this segment as well as on segments 4, 6, 8; however the character could be checked and confirmed only for segments 4, 6, 8 (Fig 5.126B-C in Adrianov and Malakhov), so the character was coded as missing data for the segment 2. The type specimens of *K. trisetosus* showed the presence of paradorsal setae on segments 2, 4, 6, 8 whereas these are absent on the remaining segments, bearing subdorsal setae actually. Even though the presence of paradorsal setae was observed in type material of *N. satyai* on segments 1, 4-8 only, we coded the character

as present on all segments 1-10 as the original description refers. The character for *P. arctous*, *P. calmani*, *P. chilensis*, *P. galtsovae*, *P. maximus*, *P. odhneri*, *K. spinosus* was coded as missing data according to the reasons given in **"Dorsal series"**.

44) **PD setae on segment 3:** (0) absent; (1) present. See information of **"Dorsal series"**. The character was coded as present in *P. oshoroensis* even though its high intraspecific variability because a seta is present in the voucher. Taxa coded as missing data or inapplicable, as well as those where the descriptions have ambiguities or the coding herein does not fit with the information given in the original descriptions is due to we follow the reasons given in character 43 **"PD setae on segment 2"**. Character for *K. distentus* was coded as inapplicable because it could not be observed in the type material.

45) **PD setae on segment 4:** (0) absent; (1) present. See information of **"Dorsal series"**. Taxa coded as missing data or inapplicable, as well as those where the descriptions have ambiguities or the coding does not fit with the information given in the original descriptions is due to we follow the reasons given in character 43 **"PD setae on segment 2"**. *Pycnophyes emarginatus* and *P. longicornis* were also coded as missing data because the presence of the setae could not be verified (same for segments 6 and 8 in *P. longicornis*). According to the literature, *K. mainensis* has protuberances on this segment (and also on segments 6 and 7), which was confirmed in additional material, as well as on segment 8.

46) **PD setae on segment 5:** (0) absent; (1) present. See information of **"Dorsal series"**. The character for *P. oshoroensis* was coded as in character 44 for the same reason. Taxa coded as missing data or inapplicable, as well as those where the descriptions have ambiguities or the coding does not fit with the information given in the original descriptions is due to we follow the reasons given in character 43 **"PD setae on segment 2"**. The illustration of *K. fimbriatus* shows a circle in paradorsal position, that could be a setae perforation site, but its absence was confirmed in the type specimens.

47) **PD setae on segment 6:** (0) absent; (1) present. See information of **"Dorsal series"**. The character for *P. oshoroensis* was coded as in character 44 for the same reason. Taxa coded as missing data or inapplicable, as well as those where the descriptions have ambiguities or the coding does not fit with the information given in the original descriptions is due to we follow the reasons given in character 43 **"PD setae on segment 2"**. *Pycnophyes longicornis* was coded as missing data because only a perforation was observed, not the seta itself (as well as on segments 4 and 8). According to the literature, *K. mainensis* has protuberances on this segment (and also on segments 2 and 7), so the character is coded as missing data.

48) **PD setae on segment 7:** (0) absent; (1) present. See information of **"Dorsal series"**. The character for *P. oshoroensis* was coded as in character 44 and for *K. mainensis* as in

character 47 for the same reasons. Taxa coded as missing data or inapplicable, as well as those where the descriptions have ambiguities or the coding does not fit with the information given in the original descriptions is due to we follow the reasons given in character 43 **"PD setae on segment 2"**. The character was coded as missing data for *P. carinatus* because it was impossible to confirm in the additional material if the seta was paradorsal or subdorsal. *Pycnophyes abyssorum* was coded as inapplicable because the presence of the character differs between the sexes.

49) **PD setae on segment 8:** (0) absent; (1) present. See information of **"Dorsal series"**. Taxa coded as missing data or inapplicable, as well as those where the descriptions have ambiguities or the coding does not fit with the information given in the original descriptions is due to we follow the reasons given in character 43 **"PD setae on segment 2"**. The character for *P. oshoroensis* was coded as in character 44 and for *P. carinatus* as in character 48 by the same reasons. Even though the original description of *K. mainensis* does not report the presence of paradorsal setae on segment 8, its presence was confirmed in the type material. Paradorsal setae are described in segments 4 and 6 for *P. beaufortensis*, however and since commonly the species with setae on these segments also bear setae on segment 8 and due to the type material was damaged on this segment, we preferred to code the character as missing data. The main text of the original description of *P. schornikovi* describes the presence of paradorsal setae on segment 8, even though the character is illustrated just like a protuberance. According to the original description, *K. stenophygus* does not have paradorsal setae on this segment, but its presence is visible in the pictures of the description (Fig. 301 Higgins 1983) and it could be confirmed in most of the type specimens (also the presence of paradorsal setae on segment 6 is visible in Fig. 300). *Pycnophyes longicornis* was coded as missing data because only a perforation was observed in the type material, not the seta itself (same for segments 4 and 6).

50) **PD setae on segment 9:** (0) absent; (1) present. See information of **"Dorsal series"**. Taxa coded as missing data or inapplicable, as well as those where the descriptions have ambiguities or the coding does not fit with the information given in the original descriptions is due to we follow the reasons given in character 43 **"PD setae on segment 2"**. The character for *P. oshoroensis* was coded as in character 44 and for *P. carinatus* as in character 48 by the same reasons. Even though the presence of the character depends on the sexes in *K. yushini* it was coded as present because the voucher bears it. The character was coded as missing data for *K. stenophygus* because a mark that could be a seta perforation sites was observed in some specimens.

51) **PD setae on segment 10:** (0) absent; (1) present. See information of**" Dorsal series"**. Taxa coded as missing data or inapplicable, as well as those where the descriptions have ambiguities or the coding does not fit with the information given in the original descriptions is due to we follow the reasons given in character 43 **"PD setae on segment**

2". According to the original description of *K. belizensis*, it bears paradorsal setae on segment 10, but direct observation of type material confirmed that these setae are present in some specimens only, and therefore it was coded as inapplicable. The character was coded as inapplicable for *K. erismatus* and *Paracentrophyes praedictus* due to its presence depends on the sexes.

52) **LD setae on segment 2:** (0) absent; (1) present. See **"Dorsal series"**. Observations of the additional material of *P. dolichurus* showed that laterodorsal setae may be present on this segment, so it was coded as inapplicable. The original drawing of *P. argentinensis* reports a pair of marks on this segment in the same position as the laterodorsal setae are located on segments 3 and 4, so we preferred to code the character as missing data for the segment 2. The presence of the character was confirmed in additional material of *P. greenlandicus*. The original description of *P. canadensis* does not report the presence of laterodorsal setae, however protuberances are illustrated in this position on most of the segments. Knowing that these protuberances may be setae but without the possibility of checking the character we preferred to code it as missing data on this segment and also on segments 3-9. The presence of the character depends on the sexes in *P. furugelmi* (same for segments 3, 5 and 7), in *P. newzealandiensis* and *P. abyssorum*, hence it was coded as inapplicable. Even though the original description of *K. distentus* reports that the character is absent in some females, all females type specimens present laterodorsal setae. Because the presence of the setae was confirmed for *P. schornikovi* in Fig. 5.110A in Adrianov and Malakhov (1999a) and for *K. belizensis* in the type specimens (on segments 2-9), the character was coded as present for these taxa, even though this information was not provided in the original description. The illustrations of *K. deirophorus* show the presence of cuticular marks in this position and in the picture 233 of the original description the insertion of a seta seems to be present. The presence of the character was verified in the type material. Laterodorsal setae are present on segments 2-9 in *K. ilyocryptus* according to Boykin (master thesis, 1965) and also these are visible in pictures 5.126A-C and 5.127A-B in Adrianov and Malakhov (1999a). Examination of *K. fimbriatus* and *K. langi* type material confirmed the presence of laterodorsal setae on segments 3, 5, 7 and 9 only (absent on segments 2, 4, 6 and 8 in *K. fimbriatus*, these are sensory spots). Moreover, the more mesial setae present on segments 2, 4, 6 and 8 in both species appear in subdorsal position, as the original descriptions report (original description of *K. fimbriatus* includes the presence of setae also on segment 5, but it is mistaken). Protuberances are described in this position on segment 2 in *K. paraneapolitanus* but it was impossible to confirm this, so we coded them as missing data. The presence of protuberances is described for *K. mainensis* on segments 2-9, and the presence of setae was confirmed by additional material. Even though the opposite is reported in the description of *K. phyllotropis*, the presence of the character was confirmed in the type material.

53) **LD setae on segment 3:** (0) absent; (1) present. *Pycnophyes arctous*, *P. calmani*, *P. canadensis*, *P. chilensis*, *P. furugelmi*, *P. maximus*, *P. odhneri*, *K. belizensis*, *K. fimbriatus*, *K. ilyocryptus*, *K. langi*, *K. mainensis*, *K. spinosus* were coded as in character 52 **"LD setae on segment 2"** following the same reasons. The observation of type materials allowed us to check the presence of laterodorsal setae on segments 3 and 4 in *P. argentinensis*, as well as its absence on this segment in *P. iniorhaptus* and its presence in *P. neuhausi* (literature does not report the presence of laterodorsal setae on segments 3-4 and 9, but laterodorsal setae are present on segments 2-9). SEM images of *K. giganteus* showed the presence of setae on this segment (according to the literature these were absent on segments 3, 5 and 9 but are actually present 2-9). The presence of the character depends on the sexes *in P. cryopygus* (type material) and *P. galtsovae*.

54) **LD setae on segment 4:** (0) absent; (1) present. *Pycnophyes arctous*, *P. argentinensis*, *P. calmani*, *P. canadensis*, *P. chilensis*, *P. galtsovae*, *P. maximus*, *P. neuhausi*, *P. odhneri*, *K. belizensis*, *K. fimbriatus*, *K. ilyocryptus*, *K. langi*, *K. mainensis*, *K. spinosus* were coded as on segment 2, character 52, following the same reasons.

55) **LD setae on segment 5:** (0) absent; (1) present. *Pycnophyes arctous*, *P. calmani*, *P. canadensis*, *P. chilensis*, *P. furugelmi*, *P. galtsovae*, *P. maximus*, *P. odhneri*, *K. belizensis*, *K. fimbriatus*, *K. ilyocryptus*, *K. langi*, *K. mainensis*, *K. spinosus* were coded as on segment 2, character 52, following the same reasons. The presence of the character depends on the sexes in *P. cryopygus*. SEM pictures of *K. giganteus* confirmed the presence of the character on this segment (according to the literature these were absent on segments 3, 5 and 9 but are actually present 2-9). The illustration of *P. schornikovi* shows the presence of laterodorsal protuberances on this segment that could be setae, but without any possibility of checking this character we preferred to code it as missing data on this segment as well as on segment 6.

56) **LD setae on segment 6:** (0) absent; (1) present. *P. arctous*, *P. calmani*, *P. canadensis*, *P. chilensis*, *P. galtsovae*, *P. maximus*, *P. odhneri*, *K. belizensis*, *K. fimbriatus*, *K. ilyocryptus*, *K. langi*, *K. mainensis*, *K. spinosus* were coded as on segment 2, character 52, following the same reasons. This character is described as absent in *K. phyllotropis*, but its presence was confirmed in the type specimens (present on segments 2-7). The presence of the character depends on the sexes in *K. yushini* but it was coded as present because the voucher has it. The character was coded as inapplicable for *P. chalgap* due to it was absent in some specimens. The character for *P. schornikovi* was coded as on segment 5, character 55.

57) **LD setae on segment 7:** (0) absent; (1) present. *P. arctous*, *P. calmani*, *P. canadensis*, *P. chilensis*, *P. furugelmi*, *P. galtsovae*, *P. maximus*, *P. odhneri*, *K. belizensis*, *K. fimbriatus*, *K. ilyocryptus*, *K. langi*, *K. mainensis*, *K. spinosus* were coded as on segment 2, character 52, following the same reasons. The presence of the character depends on the sexes in *P. cryopygus*.

58) **LD setae on segment 8:** (0) absent; (1) present. *P. arctous*, *P. calmani*, *P. canadensis*, *P. chilensis*, *P. galtsovae*, *P. maximus*, *P. odhneri*, *K. fimbriatus*, *K. ilyocryptus*, *K. langi*, *K. mainensis*, *K. spinosus* are coded as on segment 2, character 52, following the same reasons. The presence of the character depends on the sexes in *K. yushini* but it was coded as present because the voucher has it. The character was coded as inapplicable for *P. chalgap* and *K. apotomus* since the character was absent in some specimens. The illustration of *P. schornikovi* shows the presence of laterodorsal protuberances on this segment that could be setae, and fortunately it was possible to check the presence of seta in fig. 5.110B in Adrianov and Malakhov (1999a).

59) **LD setae on segment 9:** (0) absent; (1) present. *P. arctous*, *P. calmani*, *P. canadensis*, *P. chilensis*, *P. galtsovae*, *P. maximus*, *P. odhneri*, *K. fimbriatus*, *K. ilyocryptus*, *K. langi*, *K. mainensis*, *K. spinosus* were coded as on segment 2, character 52, following the same reasons. A pair of cuticular marks as seta perforation sites was observed in the voucher of *P. robustus*, but it was impossible to see the setae. Since the presence of setae for this species depends on the specimen, we preferred to code the character as missing data. Similar for *P. kielensis* and P. sp. nov. 4 (laterodorsal setae are present in some specimens only), and *P. norenburgi*, *P. abyssorum*, *K. belizensis* (the presence of the character depends on the sexes), hence the character appear as inapplicable. Even though the original descriptions of *P. borealis*, *P. greenlandicus* and *P. neuhausi* do not include information about this character, the presence of laterodorsal setae was confirmed by their type materials. The examination of *P. cryopygus* type specimens showed the absence of this character, being the structure a sensory spot actually. We could not verify the presence of laterodorsal setae in *P. iniorhaptus* on this segment and we therefore coded it as missing data. The presence of the character could not be confirmed in the SEM pictures of *K. giganteus* (according to the literature setae are absent on segments 3, 5 and 9 but are actually present 2-8).The character for *P. spitsbergensis* was coded as missing data due to some conflicts in the description between text and illustrations, and moreover, data for males are not accurate.

Lateral series: Setae in paralateral (PL) and lateroventral (LV) position were coded. Paralateral setae are located bilaterally adjacent and dorsal to the midlateral position of the segment. The position can only be observed from the dorsal side of a dorsoventrally mounted specimen. Lateroventral setae are those located bilaterally on the tergal plate, immediately adjacent to the tergosternal junction, as seen from the ventral side of a dorsoventrally mounted specimen. The lateral appendages in species of Pycnophyidae are always located in lateroventral position (except on segments 1 that are paralateral), whereas Neocentrophyidae bears them in paralateral position. Even though the descriptions of *P. arctous*, *P. calmani* (information taken from a drawing for segments 1-2, 10), *P. chilensis* and *P. maximus* do not include information about presence of any setae along the whole trunk

we preferred to code it as missing data rather than "absent" because setae are sometimes difficult to see using LM only when the specimen is not in a good condition. As for *P. furugelmi* and *P. gatlsovae*, the strange setae distribution in the remaining positions and the absence of any lateral setae, lead us to think that the information of the description may be incomplete or mistaken. The original description of *P. sculptus* does not give information about lateral setae. In all these cases, lateral setae were coded as missing data instead of absent. As for *P. newguiniensis*, the presence of lateral setae is described for segments 2, 9-10 only, which is a strange distribution (it has never been reported again for any other species by other authors and we have never seen such distribution either). Since information on its lateral seta distribution can be incomplete, we preferred to code the character as missing data for all segments, except for segments 2, 9-10. Similar for *P. schornikovi*, which original description reports the presence of lateral setae on segments 4 and 10 only. However, SEM pictures in Adrianov and Malakhov (1999a) clearly show the presence of lateral setae also on segment 2. The presence of lateral setae is reported for *K. ilyocryptus* on segments 2, 4 and 10.This mistake lead as to expect that lateral setae may be present on other segments. Therefore, we preferred to code the character as missing data for the remaining segments. In order to avoid possible conflicts regarding the homology of tubes of Cyclorhagida and setae of Pycnophyidae and Neocentrophyidae we chose to code the presence/absence of setae only, coding the absence of any structure in Cyclorhagida as 0 and the presence of tubes as missing data.

60) **Lateral appendage segment 1 (PL-LV):** (0) absent; (1) present. See "**Lateral series"**. The description of *P. odhneri* reports the absence of lateral setae. However, and since it is an old description, it is likely that the character could be present but not seen by the author. Therefore, we preferred to code the character as missing data. Even though the original description of *K. apotomus* reports the presence of lateral appendages on segment 1, it is mistaken because these are actually sensory spots. The character state was impossible to check for *Pycnophyes* 2012, and therefore it was coded as missing data. Even though the type material was studied for *P. argentinensis*, *P. ecphantor*, *P. corrugatus*, *P. emarginatus*, *P. iniorhaptus* the character state could not be confirmed, so it was coded as missing data for these species. The character was coded as inapplicable for *P. abyssorum*, for which the presence of the character depends on the sexes, and for *K. erismatus*, because the character is absent in some specimens. The description of *P. chukchiensis* reports the absence of setae, but a protuberance seems to be present in the type specimens, being impossible to confirm if it is a seta or a sensory spot, so we preferred to code it as missing data. Same coding for *K. paraneapolitanus* according to a picture in the original description. According to the original description of *K. anomalus*, there is a seta on this segment that could be located in lateral position, but we could not conclude whether it is lateral or ventral. Even tough, we previously mentioned that we coded the character as missing data for *K. ilyocryptus* (except for segments 2, 4 and 10),

the character is absent on segment 1, confirmed by fig. 5.110A in Adrianov and Malakhov (1999a).

61) **Lateral appendage segment 2 (PL-LV):** (0) absent; (1) present. See "**Lateral series"**. The original description of *P. argentinensis* reports the presence of lateroventral setae on segment 6 only, but examination of the type material confirmed its presence also on segments 2 and 4. The illustrations of *K. distentus* show ventrolateral setae instead of lateral ones on segments 2. Fortunately, the observation of the type material confirmed that these setae are actually lateroventral, and moreover, the lateroventral setae are presents on segments 4, 6, 8 and 10. The character was coded as inapplicable for *P. arctous* because its presence depends on the sexes. According to the original description the character is absent on this segment in *P. abyssorum*, but its presence it is visible in the pictures (Adrianov and Maiorova 2014) (and also it is present on segments 5, 6, 8 and 10). The character was coded as present for *K. erismatus* after observing it in the type material (the presence of these setae are not reported 8 in the original description on segments 3-6, but was confirmed by direct observation), as well as in *K. fimbriatus* (lateroventral setae are present on segments 2, 4, 6, 8 and 10 only) and *K. stenopygus* (present on segments 2, 4, 6, 8). Examination of the type material of *K. deirophorus* confirmed the presence of lateroventral setae on segments 2, 4, 6, 8 and 10, whereas those of segments 3, 5, 7 and 9 are actually ventrolateral setae (also ventrolateral setae are present on segments 6 and 8). Similar, in *K. phyllotropis*, for which a pair of setae is illustrated on the dorsal side, but is actually in lateroventral position (these setae are illustrated on the tergal plate, more laterally on segments 2, 4, 6, 8 and 9, and more dorsally on segment 10, but their real position was confirmed in the type specimens). Even though the description of *K. paraneapolitanus* reports the absence of lateral setae on this segment, it seems that a seta could be present (see Fig. 5 in Higgins and Adrianov 1991) and therefore the character was coded as missing data. Even though there are some contradictions between the main text and the illustrations of *K. trisetosus*, the presence of lateroventral setae was confirmed on segments 2-10 in type specimens.

62) **Lateral appendage segment 3 (PL-LV):** (0) absent; (1) present. See **"Lateral series"**. The character for *K. erismatus*, *K. deirophorus*, *K. fimbriatus*, *K. trisetosus* was coded following the same reasons as in character 61 **"Lateral appendage on segment 2 (PL-LV)".** The observation of type material of *P. beaufortensis* and *P. cryopygus* confirmed the absence of lateroventral setae on segment 3, described as present and without information in the original descriptions respectively.

63) **Lateral appendage segment 4 (PL-LV):** (0) absent; (1) present. See "**Lateral series"**. The character for *P. argentinensis*, *K. distentus*, *K. deirophorus*, *K. erismatus*, *K. fimbriatus*, *K. phyllotropis*, *K. stenophygus*, *K. trisetosus* was coded following the same reasons as in character 61 **"Lateral appendage on segment 2 (PL-LV)".** The presence of the character depends on the sexes in *P. abyssorum* and therefore coded as inapplicable.

64) **Lateral appendage segment 5 (PL-LV):** (0) absent; (1) present. See "**Lateral series"**. The character for *K. deirophorus* and *K. erismatus* was coded following the same reasons as in character 61 **"Lateral appendage on segment 2 (PL-LV)".** After examination of the type material of several species, we could conclude that a general mistake has been made in relation to the presence of lateroventral setae on segment 5 in the species of Pycnophyidae. Many descriptions report the presence of lateroventral setae on this segment when these are ventrolateral setae actually. For instance, this mistake was made for *P. ecphantor* (see also Fig. 138 in Higgins 1983), *P. borealis*, *K. langi*, *P. emarginatus* (see also Fig. 154 in Higgins 1983), *P. longicornis*, *P. neuhausi*, *K. apotomus* (see also Fig. 199 in Higgins 1983), *K. deirophorus* (see also Fig. 231 in Higgins 1983). Due to this is a very often mistake, we decided to code this character as missing data for all those species coded by old literature only without pictures to check the trait and for those whose type material could not be checked.

65) **Lateral appendage segment 7 (PL-LV):** (0) absent; (1) present. See **"Lateral series"**. Voucher of *P. oshoroensis* shows the absence of lateroventral setae on segment 7. This character was also absent in the type material of *P. greenlandicus*. The character for *K. deirophorus*, *K. erismatus*, *K. fimbriatus*, *K. trisetosus* was coded as in character 61 **"Lateral appendage on segment 2 (PL-LV)".** The original description of *K. ilyocryptus* reports the absence of the character on segment 7, and it was corroborated by Boykin (master Thesis, 1965) and in Fig. 5.126B in Adrianov and Malakhov (1999a).

66) **Lateral appendage segment 9 (PL-LV):** (0) absent; (1) present. This character usually appears in the Pycnophyidae literature as present. However, often the observed structure is not a setae but the protonephridial opening. This occurs in *K. deirophorus* (fig. 232 in Higgins 1983), *P. beaufortensis*, *P. ecphantor*, *P. egyptensis*, *P. emarginatus* (Sánchez, personal observations). The character for *K. deirophorus*, *K. fimbriatus*, *K. phyllotropis*, *K. trisetosus* was coded as in character 61 **"Lateral appendage on segment 2 (PL-LV)"**. The character for *P. spitsbergensis* was coded as missing data because there are some conflicts in the description between the main text and illustrations, and moreover, data for males are not very accurate. The presence of the character is related with the sexes in *P. newguiniensis* and hence coded as inapplicable.

67) **Lateral appendage segment 10 (PL-LV):** (0) absent; (1) present. The character for *K. deirophorus*, *K. distentus*, *K. fimbriatus*, *K. phyllotropis*, *K. trisetosus* was coded as in character 61 **"Lateral appendage segment 2 (PL-LV)"**. The presence of the character is related to the sexes, and hence coded as inapplicable for *P. frequens*, *P. norenburgi*, *P. newguiniensis*, *K. stenophygus*, *K. mainensis* and *Paracentrophyes praedictus*. The state of the character could not be confirmed for *Pycnophyes* 2012a, *P. ecphantor* and *K. erismatus* and in order to avoid mistakes, we preferred to code it as missing data. For *P. spitsbergensis* the character was coded as missing data since there are some conflicts in the description between the text and illustrations, and moreover, data for males are not

accurate. The presence of setae in *K. ilyocryptus* was verified in Fig. 5.127B in Adrianov and Malakhov (1999a).

Ventral series: Setae in ventrolateral (VL) and ventromedial (VM) position were coded. Ventrolateral setae are located bilaterally on the sternal plate, adjacent to the tergosternal junction. It extends over the outer longitudinal quarter of the sternal plate. Ventromedial setae are located bilaterally at or near the middle of the sternal plate, between ventrolateral and paraventral bands. Both positions can only be observed from the ventral side of a dorsoventrally mounted specimen. Even though the descriptions of *P. arctous*, *P. calmani*, *P. chilensis* and *P. maximus* do not include information about presence of any setae along the whole trunk we preferred to code it as missing data rather than "absent" because setae are sometimes difficult to see using LM only when the specimen is not in well conditions. Moreover, the descriptions of *P. sculptus*, *K. cataphractus* (except for segment 1), *K. spinosus* (except for segment 1) and *K. rabaulensis* do not give information about ventral setae. For all these cases, ventral setae were coded as missing data instead of absent. According to the original description, *P. mokievskii* has ventromedial setae only on segment 4. Due to its ventral seta distribution can be incomplete, we preferred to code the character as missing data for the remaining segments.

68) **Ventral setae on segment 1:** (0) absent; (1) present. The descriptions of *P. frequens* and *P. beaufortensis* report the presence of ventral setae or protuberances on segment 1 but its absence was confirmed in the type material. Contrarily, the presence of setae is not reported in the original description of *K. belizensis* but its presence was confirmed by the type material. Even though the lack of accurate information about ventral setae in *K. cataphractus* and *K. ilyocryptus* the absence of the character was checked in images included by Adrianov and Malakhov (1999a). The character was coded as inapplicable for *P. abyssorum* and *K. newguiniensis* because its presence depends on the sexes. The character was coded as missing data for *K. anomalus* because the character state is ambiguous in the literature.

69) **Posterior ventral sensory spot on segment 1:** (0) absent; (1) present. The character is not reported in the descriptions of *P. kielensis* and *K. mainensis* but its absence was confirmed in the additional material of *P. kielensis* and its presence in the type material of *K. mainensis*. Even though the type material of *P. beaufortensis*, *P. argentinensis* and *P. neuhausi* was examined we could not confirm the character state. The presence of the character could not be confirmed in the additional material of *K. giganteus*. The character was coded as absent for all species of *Paracentrophyes* and *Mixtophyes* because the sensory spots are located in the middle of the plate, not in the posterior margin. Data for *P. arctous*, *P. calmani*, *P. chilensis*, *P. furugelmi*, *P. galtsovae*, *P. maximus*, *P. sanjuanensis*, *P. schornikovi*, *P. sculptus*, *P. odhneri*, *K. cataphractus*, *K. spinosus* were not available, so we coded the character as missing data.

70) **Position of the posterior ventral sensory spot on segment 1:** (0) ventrolateral; (1) ventromedial. The character state could not be confirmed in *P. beaufortensis*, *P. argentinensis*, *P. neuhausi*, *K. giganteus* by the type material or additional material. Information *for P. arctous*, *P. calmani*, *P. chilensis*, *P. furugelmi*, *P. galtsovae*, *P. maximus*, *P. sanjuanensis*, *P. schornikovi*, *P. sculptus*, *P. odhneri*, *K. cataphractus*, *K. spinosus* were not found, so we coded the character as missing data.

71) **Ventrolateral setae on segment 3:** (0) absent; (1) present. The presence of these setae was confirmed in the type material for *K. deirophorus* and *K. distentus*. Despite the presence of setae is reported in the original description of *K. phyllotropis*, only marks were observed in this position in the type material. Hence, we coded the character as missing data. *Pycnophyes arctous*, *P. calmani*, *P. chilensis*, *P. maximus*, *P. sculptus*, *K. cataphractus*, *K. spinosus* and *K. rabaulensis* were coded as missing data following the reasons given in **"Ventral series"**.

72) **Ventrolateral setae on segment 4:** (0) absent; (1) present. Ventrolateral setae may be present in *P. ponticus* with absence of ventromedial setae; or setae may be absent in ventrolateral position with a setae present in ventromedial position. The voucher of *P. ponticus* has ventrolateral setae whereas the ventromedial ones are absent. For *K. deirophorus* the character was coded as inapplicable because the setae are present only in some females of the type species. Even though the absence of setae is reported in the original description of *K. distentus*, only marks were observed in this position in the type material. Hence, we coded the character as missing data. *Kinorhynchus phyllotropis*, *P. arctous*, *P. calmani*, *P. chilensis*, *P. maximus*, *P. sculptus*, *K. cataphractus*, *K. spinosus* and *K. rabaulensis* were coded as in character 71 **"Ventrolateral setae on segment 3**" following the same reasons.

73) **Ventrolateral setae on segment 5:** (0) absent; (1) present. This setae is often described in the literature as lateroventral setae, so we decided to code the character as missing data for all the species for which the character could not be confirmed in the type material or by pictures in the literature. The original description of *P. ecphantor* reports the presence of lateroventral setae on this segment but the observation of the type material confirmed that the setae are ventrolateral actually. The presence of ventrolateral setae was confirmed by the type material or additional material for *P. borealis*, *P. emarginatus*, *P. longicornis*, *P. neuhausi*, *K. apotomus*, *K. belizensis*, *K. deirophorus*, *K. distentus*, *K. erismatus*, *K. fimbriatus*, *K. giganteus*, *K. langi*, *K. mainensis*, *K. phyllotropis*, *K. stenophygus*. The character could not be observed in *P. chukchiensis* and therefore coded as missing data. *Pycnophyes arctous*, *P. calmani*, *P. chilensis*, *P. maximus*, *P. sculptus*, *K. cataphractus*, *K. spinosus* and *K. rabaulensis* were coded as in character 71 "**Ventrolateral setae on segment 3**" following the same reasons.

74) **Ventrolateral setae on segment 6:** (0) absent; (1) present. The presence of the setae was confirmed by the type material *K. deirophorus*. The character was coded as inapplicable

for *K. distentus* because the setae is present in some specimens of the type material but in other ones the setae appear in ventromedial position. The character is present in most of the type specimens of *N. satyai* and *N. intermedius* but not in all, so it was coded as inapplicable. According to the literature, females of *P. gatlsovae* has ventrolateral setae, but it is absent in males, so the character was coded as inapplicable. The character was coded as missing data for *P. mokievskii* because there is not any information in the literature.

75) **Ventrolateral setae on segment 7:** (0) absent; (1) present. Ventrolateral setae are only present in females of *P. corrugatus*, whereas males have ventromedial setae, so the character was coded as inapplicable. The presence of the character was verified by the type material of *K. deirophorus* and *K. erismatus.* The character was coded as missing data for *P. mokievskii* because there is not any information in the literature. *Pycnophyes arctous*, *P. calmani*, *P. chilensis*, *P. maximus*, *P. sculptus*, *K. cataphractus*, *K. spinosus* and *K. rabaulensis* were coded as in character 71 **"Ventrolateral setae on segment 3**" following the same reasons.

76) **Ventrolateral setae on segment 8:** (0) absent; (1) present. Females of *K. yushini* has ventrolateral setae on segment 8 but they are absent in males, as the voucher is, and hence the character was coded as absent. Original description of *P. abyssorum* does not report the presence of ventrolateral setae on this segment, but its presence was confirmed in the SEM pictures of the description. The character is present only in some type specimens of *K. deirophorus*, so the character was coded as inapplicable. The presence of the character was confirmed in type material of *K. deirophorus* despite of the original description does not report it. The character was coded as missing data for *P. mokievskii* because there is not any information in the literature. *Pycnophyes arctous*, *P. calmani*, *P. chilensis*, *P. maximus*, *P. sculptus*, *K. cataphractus*, *K. spinosus* and *K. rabaulensis* were coded as in character 71 **"Ventrolateral setae on segment 3**" following the same reasons.

77) **Ventrolateral setae on segment 9:** (0) absent; (1) present. The presence of the character was confirmed by the type specimens of *K. deirophorus*, *K. erismatus*, *K. langi* and *K. mainensis*. No data for *P. furugelmi*, coded as missing data. *Pycnophyes arctous*, *P. calmani*, *P. chilensis*, *P. maximus*, *P. sculptus*, *K. cataphractus*, *K. spinosus* and *K. rabaulensis* were coded as in character 71 **"Ventrolateral setae on segment 3**" following the same reasons.

78) **Ventrolateral setae on segment 10:** (0) absent; (1) present. Often the old descriptions report the presence of two pairs of "lateral setae" but it is not specified if both setae are located in the dorsal or ventral plates or one in the dorsal and one in the ventral side. Therefore and in order to avoid mistakes, we coded the character as missing data for the species for which the character could not be checked in the type material and the position of the setae is not specified in the literature. *Pycnophyes arctous*, *P. calmani*, *P.*

chilensis, *P. maximus*, *P. sculptus*, *K. cataphractus*, *K. spinosus* and *K. rabaulensis* were coded as in character 71 **"Ventrolateral setae on segment 3**" following the same reasons.

79) **Ventromedial setae on segment 3:** (0) absent; (1) present. Females of *P. flaveolatus* and *P. dentatus* have ventromedial setae, but the character is absent in males, as the vouchers are. Character for *P. abyssorum* and *K. erismatus* was coded as inapplicable because its presence depends on the sexes. *Pycnophyes greenlandicus* has ventromedial setae instead of ventrolateral as the drawing in the original description shows. Its presence was confirmed in the type specimens of *K. belizensis* and its absence in *K. distentus*. *Pycnophyes arctous*, *P. calmani*, *P. chilensis*, *P. maximus*, *P. mokievskii*, *P. sculptus*, *K. cataphractus*, *K. spinosus* and *K. rabaulensis* were coded as missing data following the reasons given in **"Ventral series"**.

80) **Position of the ventral sensory spots related to the ventromedial setae (for most of the segments**): (0) laterally; (1) mesially. The character for *K. yushini*, *P. dolichurus* and *K. deirophorus* was coded as inapplicable since the species does not have ventromedial setae. When the species has more than one pair of ventromedial setae and the sensory spots appear located between them, the character was coded as inapplicable, as in *P. carinatus*, *P. belizensis* and *K. trisetosus*. The character was coded as inapplicable for those species with more than one pair of sensory spots by segment and the ventromedial setae located between them, as in *K. fimbriatus*, *K. langi* and *K. mainensis*. *Kinorhynchus distentus* has sensory spots located laterally and mesially to the ventromedial setae in the same number of segments, so the character was coded as inapplicable. The character was coded as missing data when the original description does not report information about ventromedial setae or sensory spots: *Pycnophyes arctous*, *P. calmani*, *P. chilensis*, *P. furugelmi*, *P. galtsovae*, *P. maximus*, *P. mokievskii*, *P. newguiniensis*, *P. odhneri*, *P. sanjuanensis*, *P. sculptus*, *K. anomalus*, *K. cataphractus*, *K. spinosus* and *K. rabaulensis.*

81) **Ventromedial setae on segment 4:** (0) absent; (1) present. The absence of the character was confirmed in the type specimens of *P. neuhausi* even though the original description reports that ventromedial setae are present. The presence of the character was verified in type specimens for *K. belizensis*. The character was coded as absent for *K. langi* because the observation of the type material confirmed that the structure is a sensory spot actually. *Pycnophyes abyssorum*, *K. erismatus*, *P. arctous*, *P. calmani*, *P. chilensis*, *P. maximus*, *K. cataphractus*, *K. spinosus* and *K. rabaulensis* were coded as in character 79 **"Ventromedial setae on segment 3**" following the same reasons.

82) **Ventromedial setae on segment 5:** (0) absent; (1) present. *Pycnophyes neuhausi*, *K. belizensis* and *K. erismatus* were coded as in character 81 **"Ventromedial setae on segment 4"** following the same reasons. *Pycnophyes abyssorum*, *K. erismatus*, *P. arctous*, *P. calmani*, *P. chilensis*, *P. maximus*, *P. mokievskii*, *K. cataphractus*, *K. spinosus* and *K.*

rabaulensis were coded as in character 79 **"Ventromedial setae on segment 3"** following the same reasons.

83) **Ventromedial setae on segment 6:** (0) absent; (1) present. The presence of the character was confirmed in type specimens of *P. neuhausi*. Only some specimens of *K. distentus* have ventromedial setae and the character state depends on the sexes in P. galtsovae, so the character was coded as inapplicable. Data for *K. langi* was coded as absent because examination of the type material revealed that the structure is a sensory spot. *Pycnophyes abyssorum*, *P. arctous*, *P. calmani*, *P. chilensis*, *P. maximus*, *P. mokievskii*, *K. cataphractus*, *K. spinosus* and *K. rabaulensis* were coded as in character 79 **"Ventromedial setae on segment 3"** following the same reasons.

84) **Ventromedial setae on segment 7:** (0) absent; (1) present. The presence of the ventromedial setae was observed in some specimens of *P. corrugatus* and *P. cryopygus*, but not in all, being the setae sometimes displaced to other position, so the character was coded as inapplicable. The ventral setae of *P. furugelmi* seem to be a bit displaced of the ventromedial position according to the drawing of the original description, but it was impossible to confirm in the type material, so the character was coded as inapplicable. The presence of the character was confirmed in the type specimens of *P. neuhausi*. *Pycnophyes abyssorum*, *P. arctous*, *P. calmani*, *P. chilensis*, *P. maximus*, *K. mokievskii*, *K. cataphractus*, *K. spinosus* and *K. rabaulensis* were coded as in character 79 **"Ventromedial setae on segment 3"** following the same reasons.

85) **Number of sensory spots on segment 8 in subdorsal and laterodorsal position before the laterodorsal setae:** (0) 1; (1) 2; (2) 3 or more. No data were available in the old descriptions, hence, when the type material was not available or it was damaged, the character as missing data. The character was coded as inapplicable for *P. carinatus* and *K. trisetosus* due to this species has two pairs of laterodorsal setae with a sensory spots between them.

86) **Ventromedial setae on segment 8:** (0) absent; (1) present. Even though the drawing in the original description of *P. longicornis* shows the ventral setae displaced to the ventrolateral position, the displacement is minimal, and the setae are still in ventromedial position. The presence was confirmed in the type species of *P. neuhausi*. The presence of these setae is reported in the original description of *K. phyllotropis* but the study of type material confirmed that the character is absent. The presence of the character was confirmed in the type specimens of *P. neuhausi. Pycnophyes abyssorum*, *P. arctous*, *P. calmani*, *P. chilensis*, *P. maximus*, *K. mokievskii*, *K. cataphractus*, *K. spinosus* and *K. rabaulensis* were coded as in character 79 **"Ventromedial setae on segment 3"** following the same reasons.

87) **Ventromedial setae on segment 9:** (0) absent; (1) present. Females of *P. dentatus* has ventromedial setae, whereas the character is absent in males, and hence the character

was coded as present for the female voucher. The character was coded as inapplicable for *P. almansae* since the setae are present in most of the studied specimens but not in all. Despite of the information given in the original description, the presence of the character was confirmed in the type specimens of *P. argentinensis* and its absence in *K. mainensis*. The ventral setae in *P. cryopygus*, *K. apotomus* and *K. belizensis* (only in females) are a bit displaced, but they are still located in ventromedial position. The presence was confirmed in the type species of *P. neuhausi*. The character depends on the sexes in *K. deirophorus* and *K. stenophygus*, so it was coded as inapplicable. *Pycnophyes abyssorum*, *P. arctous*, *P. calmani*, *P. chilensis*, *P. maximus*, *K. mokievskii*, *K. cataphractus*, *K. spinosus* and *K. rabaulensis* were coded as in character 79 "**Ventromedial setae on segment 3**" following the same reasons.

88) **Number of ventral sensory spots on segment 9:** (0) 1; (1) 2; (2) 3. Often not reported in the old literature and therefore we coded it as missing data when the type material was not available to check the character. The presence of two pairs of sensory spots was observed in the type material or additional specimens of *P. beaufortensis*, *P. cryopygus* and *K. giganteus*. A single pair was observed in *K. belizensis*, whereas two pairs are drawing in the original description (the second pair is a setae actually). Three pairs were observed in *K. langi*, *K. mainensis* and *K. phyllotropis*.

89) **Longitudinal cuticular thickening on segment 10:** (0) absent; (1) present. The cuticle may form a thick fold, often longitudinally, close to the lateral margins of the sternal plates. The drawings in the original description of *P. corrugatus* and *K. apotomus* show longitudinal lines close to the lateral margins of the segment 10, but these are pectinate fringe actually.

90) **Pairs of paradorsal setae:** (0) absent; (1) present. Even though the presence of paired paradorsal setae depends on the specimen in *P. oshoroensis* it was coded as present because the voucher has paired setae. The character was coded as inapplicable for *P. communis* and *K. belizensis* because in both cases the specimens often have unpaired setae but we found some specimens with paired paradorsal setae. The character was coded as absent in *K. trisetosus* due to the paired setae are those located in subdorsal position, not the paradorsal ones. Even though we could not verified the presence of pairs of paradorsal setae on any segment in *N. satyai* due to the specimens were damaged, we coded the character as the literature reports. Species without information in the literature about paradorsal setae were coded as missing data (see **"Dorsal series"**).

91) **Presence of pairs of paradorsal setae by segments:** (0) even segments only; (1) uneven segments only; (2) both. The character was coded following the reasons given for the characters related to the paradorsal setae by each segment (see **"Dorsal series"** and characters 42-51).

92) **All ventral setae in the same position aligned along the trunk (excluding the segments 1 and 2):** (0) yes; (1) no. The character was coded following the reasons given for the characters related to the ventrolateral and ventromedial setae by each segment (see **"Ventral series"** and characters 71-79, 81-84, 86-87). The character was coded as inapplicable for *P. almansae* because a shift was observed in most of the specimens but not in all. The displacement occurs only in females in *K. belizensis* and *K. stenophygus*, so the character was coded as inapplicable.

93) **Location of the displaced setae:** (0) 3-6; (1) 7-9; (2) both. The character was coded as inapplicable in *P. almansae*, *K. belizensis* and *K. stenophygus* following the same reasons as in character 92 **"All ventral setae in the same position aligned along the trunk"**.

94) **Ventral setae on segment 7 displaced to others ventromedial setae:** (0) no; (1) yes. The character was coded as inapplicable for *P. corrugatus*, *P. cryopygus* and *K. distentus* because the shift occurs in some specimens only.

95) **Ventral Setae on segment 8 displaced related to others ventromedial setae**: (0) no; (1) yes. The character was coded as inapplicable for *P. almansae* and *K. distentus* because a shift was observed in most of the specimens but not in all.

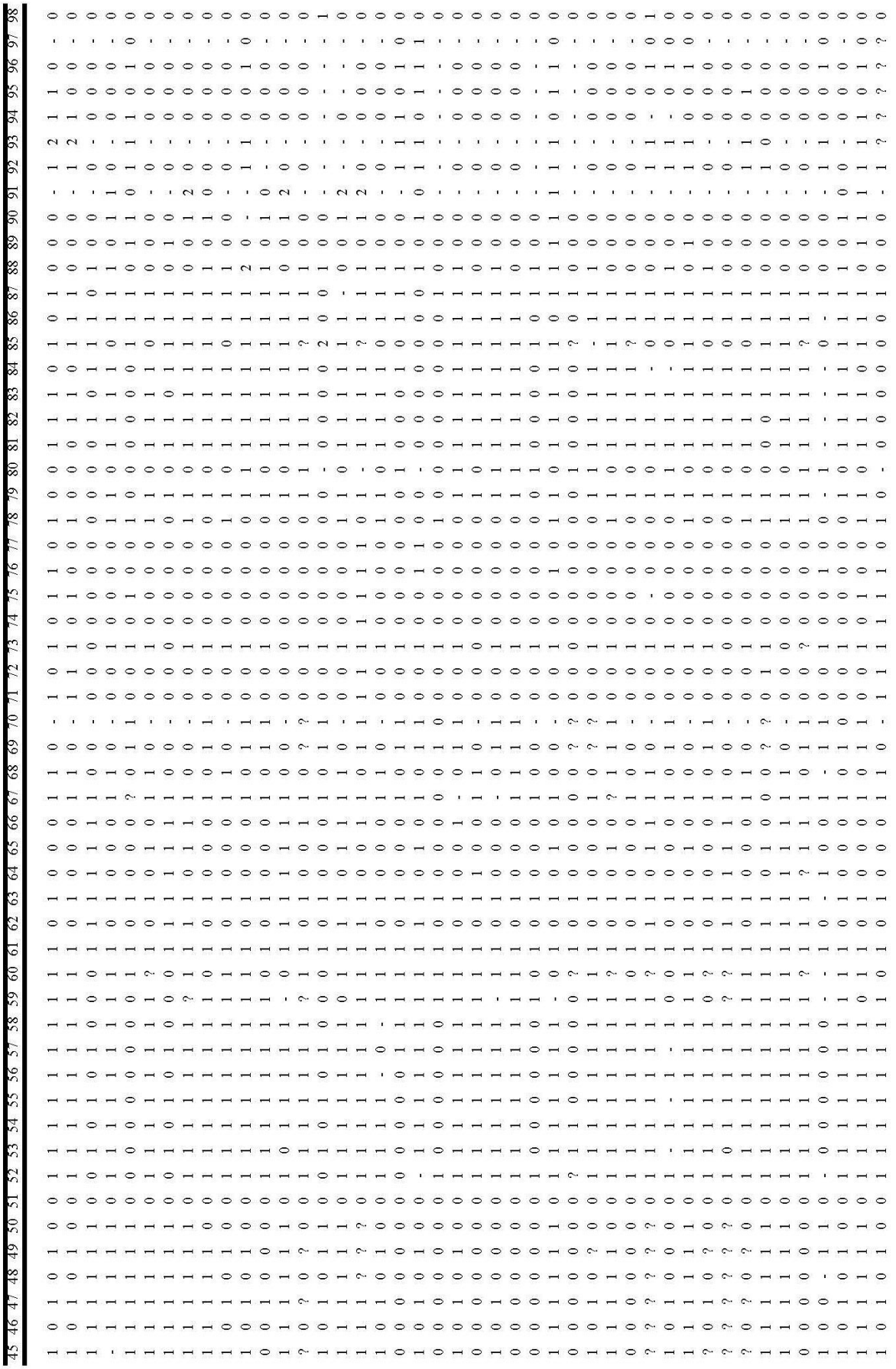
96) **Ventral Setae on segment 9 displaced related to others ventromedial setae:** (0) no; (1) yes. The character was coded as inapplicable in *P. almansae*, *K. belizensis* and *K. stenophygus* following the same reasons as in character 92 **"All ventral setae in the same position aligned along the trunk"**.

97) **Shift ventral setae on segment 9:** (0) mesial; (1) lateral. The character was coded as inapplicable in *P. almansae*, *K. belizensis* and *K. stenophygus* following the same reasons as in character 92 **"All ventral setae in the same position aligned along the trunk"**.

98) **Number of ventral sensory spots for most of the segments 3-8:** (0) 1; (1) 2. Even though the original description of *K. belizensis* reports the presence of one pair of sensory spots on segments 2-5 and two pairs on segments 6-9 the observation of the type material confirmed that there is a single pair on each segment. For species without information about sensory spots in the description and those without available type material, the character was coded as missing data (see characters 80 and 88 **"Position of the ventral sensory spots related to the ventromedial setae (for most of the segments)"** and **"Number of ventral sensory spots on segment 9"**).

I.III. MORPHOLOGICAL MATRIX OF PYCNOPHYIDAE AND NEOCENTROPHYIDAE

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44
Pycnophyidae																																												
Pycnophyes rugosus	0	1	0	2	1	1	0	1	0	0	0	0	1	0	3	1	1	0	0	-	0	0	0	0	2	1	5	1	1	1	3	0	0	0	0	0	0	0	0	0	0	0	1	0
Pycnophyes ponticus	0	1	0	1	2	1	0	1	0	0	0	0	3	0	3	1	1	0	0	-	0	0	0	0	2	1	5	1	1	1	3	0	0	0	0	0	0	0	0	0	0	0	1	0
Pycnophyes flaveolatus	0	0	0	1	1	1	0	1	0	0	0	0	1	0	3	1	1	0	0	-	0	0	1	0	2	1	6	0	0	1	1	0	0	0	0	0	1	1	1	0	0	0	1	1
Pycnophyes oshoroensisis	0	0	0	2	2	1	0	1	0	0	0	0	3	0	3	1	1	0	0	-	0	0	1	0	2	1	2	1	1	1	3	0	0	0	0	0	0	0	0	0	0	0	-	1
Pycnophyes sp. 2012a	0	1	0	2	1	1	0	1	0	0	0	0	3	0	3	1	1	0	0	-	0	0	1	0	0	?	1	0	?	?	1	3	0	0	0	0	0	0	0	0	0	0	1	1
Pycnophyes sp. 2012b	?	?	0	5	1	1	0	1	0	0	0	0	3	0	3	1	1	0	0	-	0	0	1	0	2	1	?	1	1	1	3	0	0	0	0	0	1	1	1	0	0	0	1	1
Pycnophyes dentatus	1	0	0	0	2	1	1	0	1	0	0	0	3	0	3	1	1	0	0	-	0	0	1	0	2	1	6	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1
Pycnophyes robustus	0	0	0	0	2	1	1	0	1	0	0	0	3	0	3	1	1	0	0	-	0	0	1	0	2	1	6	2	2	1	3	0	0	0	0	0	0	0	0	0	0	0	1	1
Pycnophyes zelinkaei	0	0	0	0	3	1	1	0	1	0	0	0	3	0	3	1	1	0	0	-	0	0	1	0	2	1	0	1	1	1	3	0	0	0	0	0	0	0	1	0	0	0	1	0
Pycnophyes tuberiferus	0	0	0	0	2	2	1	0	1	0	0	0	3	0	3	1	1	0	0	-	0	0	1	0	2	1	2	1	1	1	3	0	0	0	0	0	0	0	0	0	0	0	1	0
Pycnophyes communis	1	0	0	0	2	1	1	0	1	0	0	0	3	0	3	1	1	0	0	-	0	0	1	1	2	1	0	3	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pycnophyes greenlandicus	0	0	0	0	5	2	1	0	1	0	0	0	3	0	3	1	1	0	0	-	0	0	1	1	2	1	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Pycnophyes kielensis	?	?	0	2	?	1	0	1	0	0	0	0	1	0	3	1	1	0	0	-	0	0	1	0	2	1	6	0	?	0	1	1	1	0	0	0	4	4	4	3	0	0	0	1	1
Klnorhynchus gigantens	-	-	0	3	1	1	0	1	0	0	0	0	3	0	3	1	1	0	0	-	0	0	1	1	2	1	0	?	3	1	?	0	0	0	0	0	4	4	4	3	0	0	0	1	0
Klnorhynchus yushini	-	-	1	2	2	1	0	1	0	0	0	0	3	0	3	1	1	0	0	-	0	?	1	0	0	1	1	?	?	1	0	1	1	1	1	1	1	1	1	1	2	1	0	0	0
Pycnophyes almansae	0	0	0	0	2	1	1	0	1	0	0	0	3	0	3	1	1	0	0	-	0	0	1	0	2	1	6	3	3	1	3	0	0	0	0	0	0	0	0	0	0	0	1	1
Pycnophyes carinatus	0	0	0	1	2	1	0	1	0	0	0	0	1	0	3	1	1	0	0	-	0	0	1	0	2	1	0	1	1	0	1	1	1	1	1	1	1	1	1	0	0	0	1	1
Pycnophyes chalgap	0	0	0	0	2	1	1	0	1	0	0	0	3	0	3	1	1	0	0	-	0	0	1	?	2	1	3	1	1	1	3	0	0	0	4	4	4	4	3	0	0	0	1	0
Pycnophyes cristatus	0	0	0	0	5	1	1	0	1	0	0	0	0	0	3	1	1	0	0	-	0	0	1	0	2	1	0	1	1	1	1	1	1	1	1	1	1	1	1	2	1	0	0	0
Pycnophyes dolichurus	0	1	0	0	3	2	1	0	1	0	0	0	0	0	3	1	1	0	0	-	0	0	0	0	2	1	0	1	1	1	3	0	0	0	0	0	0	1	1	0	0	0	1	1
Pycnophyes farinellii	1	0	0	4	1	1	1	0	1	0	0	0	3	0	3	1	1	0	0	-	0	0	0	?	1	0	1	0	1	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0
Pycnophyes frequens	1	0	0	4	1	1	0	1	0	0	0	0	?	0	3	1	1	0	0	-	0	0	1	1	2	1	1	3	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pycnophyes lageria	0	0	0	1	2	1	0	1	0	0	0	0	3	0	3	1	1	0	0	-	0	0	1	0	0	1	1	1	1	1	3	3	3	4	4	4	4	4	3	0	0	0	0	0
Pycnophyes norenburgi	1	0	0	3	1	1	0	1	0	0	0	0	3	0	3	1	1	0	0	-	0	0	1	1	2	1	0	?	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pycnophyes nubilis	0	0	0	0	4	1	1	0	1	0	0	0	1	0	3	1	1	-	0	-	0	0	-	0	?	1	0	1	1	1	1	1	1	1	1	1	1	1	2	1	1	0	0	0
Pycnophyes smang	0	0	0	0	5	1	1	0	1	0	0	0	0	0	3	0	3	1	1	0	0	-	0	0	1	1	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pycnophyes sp. nov. 4	0	1	0	1	1	1	1	0	1	0	0	0	0	0	3	0	3	1	1	0	0	-	0	0	?	1	1	3	3	1	3	0	0	0	0	0	0	0	0	0	0	0	1	1
Pycnophyes argentinensis	0	0	0	4	2	1	0	1	0	0	0	0	3	0	3	1	1	-	0	-	0	0	-	0	2	1	0	1	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pycnophyes beaufortensis	1	0	0	3	?	1	0	1	0	0	0	0	1	0	3	1	1	0	0	-	0	0	1	0	?	1	0	3	3	?	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pycnophyes ecphantor	0	1	0	1	?	1	0	1	0	0	0	0	3	0	3	1	1	0	0	-	0	0	0	0	2	1	0	3	?	?	3	3	3	4	4	4	4	4	3	0	0	0	1	0
Pycnophyes borealis	0	0	0	5	1	1	0	1	0	0	0	0	3	0	3	1	1	0	0	-	0	0	1	0	2	1	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Pycnophyes corrugatus	0	1	0	3	1	1	0	1	0	0	0	0	3	0	3	1	1	0	0	-	0	0	1	0	2	1	1	3	?	1	3	3	3	4	4	4	4	4	3	0	0	0	?	?
Pycnophyes cryopygus	0	0	0	0	4	2	1	0	1	0	0	0	0	0	3	1	1	0	0	-	0	0	1	0	2	1	0	?	1	1	1	1	1	1	1	1	1	1	1	2	0	0	0	0
Pycnophyes egyptensis	0	0	0	0	2	1	1	0	1	0	0	0	0	0	3	1	1	0	0	-	0	0	0	0	2	1	0	3	3	1	3	0	0	0	0	0	0	0	0	1	0	0	1	1
Pycnophyes emarginatus	0	0	0	0	2	2	1	0	1	0	0	0	0	0	3	1	1	0	0	-	0	0	0	0	2	1	0	1	1	1	3	0	0	0	0	0	4	4	3	0	0	0	0	0
Pycnophyes iniorhaptus	0	0	0	0	2	1	1	0	1	0	0	0	0	0	3	1	1	0	0	-	0	0	0	0	2	1	0	0	0	?	-	0	0	0	0	0	0	0	0	0	0	0	?	?
Pycnophyes longicornis	0	1	0	1	2	1	0	1	0	0	0	?	?	0	3	1	1	0	0	-	0	0	0	0	2	1	0	3	3	0	3	1	1	1	1	1	1	1	1	0	0	-	0	0
Pycnophyes neuhausi	0	0	0	0	2	2	1	0	1	0	0	0	0	0	3	1	1	0	0	-	0	0	1	0	2	1	0	3	3	1	0	0	0	0	0	0	0	0	0	0	0	1	1	1
Pycnophyes australensis	1	0	0	0	2	2	1	0	1	0	0	0	0	0	3	1	1	0	0	-	0	0	1	0	2	1	1	?	?	1	3	0	0	0	0	0	0	0	0	0	0	0	1	1
Pycnophyes chukchiensis	0	0	0	0	3	1	1	0	1	0	0	0	3	0	3	1	1	0	0	-	0	0	1	0	2	1	0	?	?	1	-	1	1	1	1	1	1	1	1	2	1	0	0	0
Pycnophyes abyssorum	0	0	0	0	4	1	1	0	1	0	0	0	0	0	3	1	1	0	0	-	0	0	1	0	2	1	0	1	1	1	1	1	1	1	1	1	1	1	1	2	1	0	1	1
Pycnophyes aulacodes	1	0	0	0	4	1	1	0	1	0	0	0	0	0	3	1	1	0	0	-	0	0	1	1	2	1	0	3	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pycnophyes pardosi	0	0	0	0	2	1	1	0	1	0	0	0	0	0	3	1	1	0	0	-	0	0	1	1	2	1	2	3	3	1	3	3	3	4	4	4	4	4	3	0	0	0	1	1
Pycnophyes sp. nov.5	0	1	0	0	2	2	1	0	1	0	0	0	0	0	3	1	1	0	0	-	0	0	0	0	0	?	1	5	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0



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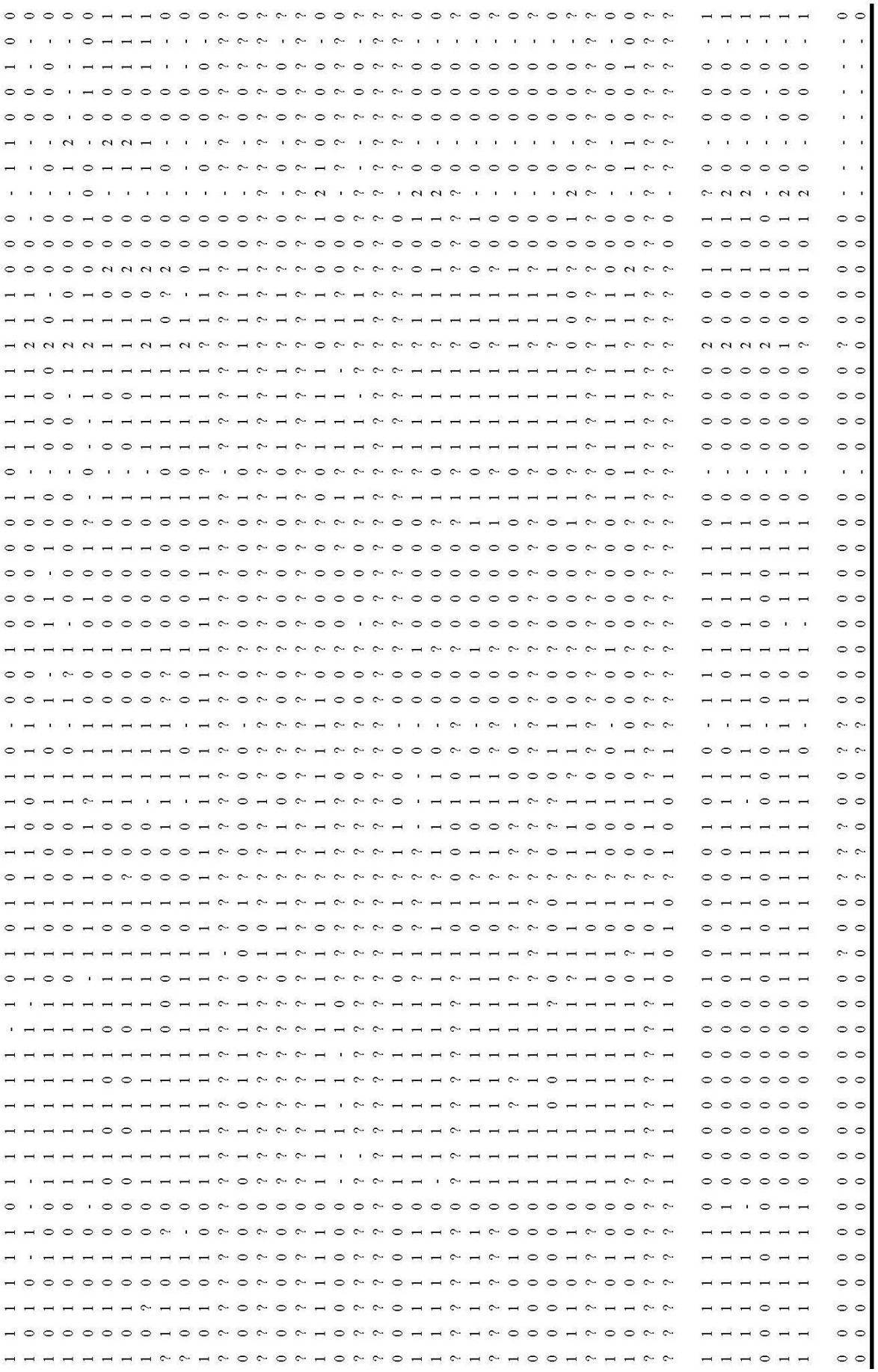


RESULTS

Appendix II

Geographical distribution

II.I. NEW DATA ON GEOGRAPHICAL DISTRIBUTION WITH SPECIAL FOCUS ON THE IBERIAN PENINSULA

Only a few regions, such as the North American East Coast, the European West Coast and the Mediterranean, have been extensively surveyed and therefore can be considered relatively well-investigated. However, even throughout these areas, the discovery of new species or new cites are still frequent. This additional data aims, firstly, to enlarge and complement the previous biogeographical study in the Iberian Peninsula carried out by Sánchez et al. (2012), providing new reports in additional localities, mostly from the southern coast of the Iberian Peninsula, Ceuta and a several points from the Northeast and Northwest. Secondly, we want to include herein the results obtained from a sampling campaign in the Gulf of Naples. Most of this area was previously sampled by Zelinka (1928) but we extend the limits of the sampling area, including new localities never sampled before. Lastly, we compile here new data from several additional regions around the world: Espegrend (Norway), Greenland (Denmark), Maine, Massachusetts and Florida (USA), Singapore, Bocas del Toro and Naos (Panana).

MATERIAL AND METHODS

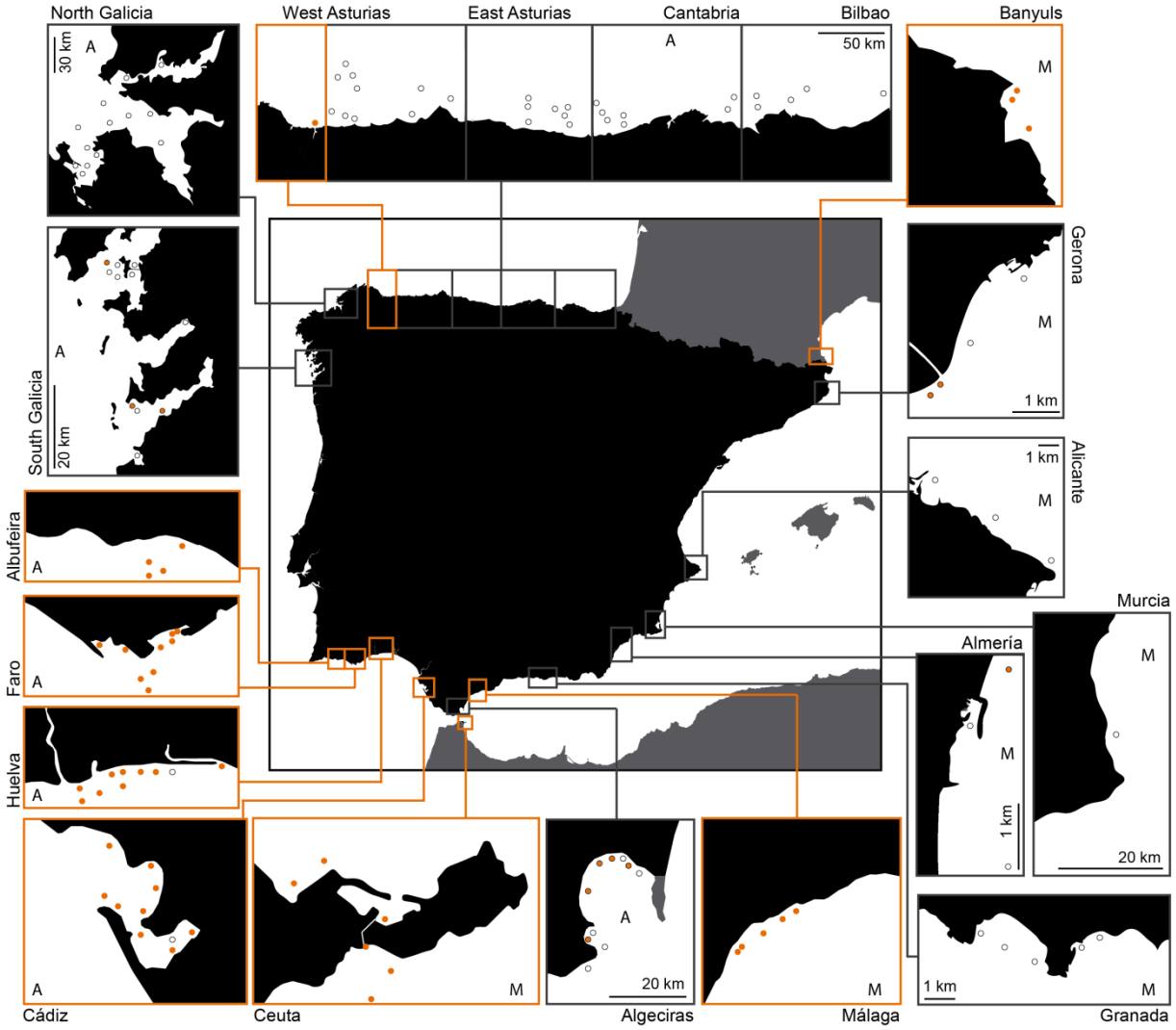
Data included herein belong to samples taken in Asturias, Albufeira, Faro, Huelva and Cádiz (Atlantic coasts of the Iberian Peninsula); and Ceuta, Málaga and Banyuls (Mediterranean coast of the Iberian Península); Naples (Italy); Espegrend (Norway); Greenland (Denmark); East Coast of USA (Maine, Massachusetts, Florida); Singapore, Bocas del Toro (Caribbean Sea) and Naos (Pacific Ocean) (Panama). Samples were collected using a Higgins Meiobenthic Dredge or a Van been dredge (Higgins, 1964;, Higgins and Thiel, 1988). The specimens were sorted, fixed and examined as is referred in the *Material and Methods* section of this Thesis.

As for the samples of the Iberian Peninsula, data from the study carried out by Sánchez et al. (2012) were included in the new analyses and discussed herein in order to have a more complete picture about the Iberian kinorhynch fauna. Therefore, the sampled localities along the Iberian coasts leave grouped into 16 areas, nine along the Atlantic coast and seven along the Mediterranean one: Bilbao, Cantabria (Castro-Urdiales, Santoña, Comillas and San Vicente de la Barquera), East Asturias (Deva mouth and Llanes), West Asturias (Cabo Peñas, Cudillero and Navia), North Galicia (Ría de Ares, Ría de Ferrol and Ría de La Coruña), South Galicia (Ría de Arosa, Ría de Pontevedra and Ría de Vigo), Gulf of Cádiz (Huelva and Cádiz), Algeciras Bay, Ceuta, Málaga, Granada (Almuñécar), Almería (Garrucha), Murcia (Isla Grosa), Valencia (Denia), Gerona (Blanes) and Banyuls (France) (Figs.23-24 and Table 5).

For the purpose of this appendix we follow the general biogeographic approach of including Algeciras Bay and Ceuta in the Atlantic sector.

RESULTS

Iberian Peninsula: Combining the new data with those gathered in Sánchez et al. (2012) a total of 31 species belonging to 11 genera were found from 122 sampling localities along the coasts of the Iberian Peninsula. 16 species are cyclorhagids and 15 are allomalorhagids. Within the Allomalorhagida, 7 new species and 8 new citations were found as a result of the current study. Information on the enlarged distribution of the Iberian kinorhynchs is provided in Figure 23 and Table 5.



Fiure 23. Map showing collecting areas and localities (close-up in the insets) yielding kinorhynchs along the Iberian Peninsula. Orange circlets mark new sampling localities after Sánchez et al. (2012), orange circlets with a black outline mark new data of sampling localities included in the referred study; white circlets mark the sampling points included in Sánchez et al. (2012). Abbreviations: M, Mediterranean Sea; A, Atlantic Sea.

Table 5. New collecting data and Allomalorhagida species from the Iberian Peninsula and Ceuta after Sánchez et al. (2012). Collectors: NS (Nuria Sánchez), FP (Fernando Pardos), MH (María Herranz), JB (Jesús Benito), RN (Ricardo Neves). Dracoderes

Locality	Date	Sample	Coordinates	Depth	Sediment	Species
Eo River	01.07.2007	5	43°32,0'N 7° 01,6' W	2	Muddy sand	*Pycnophyes dentatus*
Arosa	24.02.2009	2	42°33,242'N 8°56,357'W	28	Mud	*Pycnophyes flaveolatus*
Vigo	25.04.2009	6	42°14,089'N 8°45,564'W	21	Mud	*Pycnophyes flaveolatus*
	24.09.2009	8 (2)	42° 14,640'N 8° 51,487'W	23	Medium sand	*Pycnophyes flaveolatus*
		10 (6)	42° 14,089'N 8° 45,564'W	20	Mud	*Pycnophyes flaveolatus*
Albufeira	28.10.2013	1	36° 57,664'N 8° 09,637'W	45	Mud	*Dracoderes gallaicus*
		2	36° 59,866'N 8° 09,313'W	35	Coarse sand with shell gravel	*Pycnophyes communis* *Pycnophyes robustus* *Dracoderes gallaicus*
Faro	16.12.2012	2	37° 07,714'N 8° 36,329'W	Intertidal	Mud with *Zostera*	*Pycnophyes* sp3
	21.10.2013	1	36° 54,395'N 7° 53,970'W	96	Mud	*Pycnophyes robustus* *Dracoderes gallaicus*
		2	36° 59,311'N 7° 53,260'W	7	Mud with Shell gravel	*Dracoderes gallaicus*
		3	36° 56,863'N 7° 52,525'W	66	Mud	*Pycnophyes dentatus* *Pycnophyes communis* *Dracoderes gallaicus*
		4	36° 57,125'N 7° 52,391'W	44	Mud	*Pycnophyes communis*
	26.10.2013	3	37° 01,743'N 7° 47,924'W	4	Mud with *Zostera*	*Pycnophyes dentatus*
		4	37° 00,180'N 7° 49,339'W	2	Mud with *Ulva*	*Pycnophyes dentatus*
Huelva	11.04. 2011	1	37° 12,320'N 7° 20,534'W	4	Shell gravel	*Pycnophyes communis*
		2	37° 11,940'N 7° 21,236'W	2	Mud with shells	*Pycnophyes dentatus*
		3	37° 10,963'N 7° 16,549'W	11	Muddy sand	*Pycnophyes communis* *Pycnophyes dentatus*
		4	37° 11,527'N 7° 14,601'W	12	Muddy sand	*Pycnophyes dentatus*

		6	37° 11,887'N 7° 13,019'W	6,8	N/A	*Pycnophyes communis* *Pycnophyes dentatus*
	12.04.2011	3	37° 08,639'N 7° 21,176'W	12	Fine sand	*Pycnophyes dentatus*
		4	37° 08,324'N 7° 20,308'W	15	Muddy sand	*Pycnophyes communis* *Pycnophyes dentatus*
		5	37° 10,586'N 7°18,522'W	10	Medium sand	*Pycnophyes communis* *Pycnophyes dentatus*
Cádiz	10.11.2011	1	36° 33,755'N 6°18,500'W	13	Muddy sand	*Pycnophyes communis* *Pycnophyes dentatus* *Pycnophyes lageria*
		2	36° 35,791'N 6° 17,888'W	10	Mud	*Pycnophyes communis* *Pycnophyes dentatus*
		3	36° 34,117'N 6° 15,141'W	7	Sandy mud	*Pycnophyes communis* *Pycnophyes dentatus*
		4	36° 32,761'N 6°16,268'W	11	Coarse sand	*Pycnophyes almansae* *Pycnophyes communis* *Pycnophyes dentatus*
	11.11.2011	3	36° 29,798'N 6°12,871'W	1	Mud with *Zostera*	*Pycnophyes lageria*
Algeciras	07.02.2011	2A	36°05,354'N 5°26,170'W	08-10	Muddy sand	*Pycnophyes flaveolatus*
	08.02.2011	1B	36°09,272'N 5°26,296'W	12	Mud	*Pycnophyes dentatus*
		2B	36°10,348'N 5°26,464'W	15-17	Fine mud	*Pycnophyes flaveolatus*
		3B	36°10,583'N 5°24,620'W	25	Fine mud	*Pycnophyes flaveolatus* *Pycnophyes cf. ponticus*
		5B	36°09,630´N 5°22,256'W	12	Mud	*Pycnophyes dentatus* *Pycnophyes robustus*
Ceuta	22.05.2013	2	35° 53,14´N 5° 19,06´W	29	coarse sand+shell gravel+calcareous algae	*Pycnophyes almansae*
		4	35° 52,18´N 5° 20,25´W	20	Muddy sand	*Pycnophyes dentatus* *Pycnophyes zelinkaei*
Málaga	02.05.2012	1	36° 25,150'N 5° 09,830'W	35	Sandy mud	*Pycnophyes almansae* *Pycnophyes dentatus*
		2	36° 26,235'N 5°04,740'W	20	Muddy sand	*Pycnophyes aulacodes* *Pycnophyes dentatus* *Pycnophyes robustus* *Pycnophyes zelinkaei*
		3	36° 24,139'N 5° 09,497'W	35	Sandy mud	*Pycnophyes aulacodes* *Pycnophyes dentatus*

						Pycnophyes flaveolatus *Pycnophyes robustus* *Pycnophyes zelinkaei*
		4	36° 24,139'N 5° 09,497'W	36	Sandy mud	*Pycnophyes aulacodes* *Pycnophyes carinatus* *Pycnophyes dentatus* *Pycnophyes flaveolatus* *Dracoderes gallaicus*
		5	36°23,452'N 5° 10,796'W	36	Mud	*Pycnophyes aulacodes* *Pycnophyes dentatus* *Pycnophyes flaveolatus* *Dracoderes gallaicus*
Garrucha	25.03.1997	2	37°11'40,68''N 1°48'1,81''W	8	N/A	*Pycnophyes cf. ponticus*
Blanes	24.03.1999	5	41°39,386'N 2° 48,341'W	55,4	Dentalium sand (Coarse sand)	*Pycnophyes robustus*
		6	41°39,420'N 2°47,970'W	30	Fine sand	*Pycnophyes robustus*
Banyuls	11.09.2013	1	42°29,108'N 3°09,044'E	35	Mud	*Pycnophyes communis* *Pycnophyes dentatus* *Dracoderes gallaicus*
		2	42°29,898'N 3°08,996'E	35	Mud	*Pycnohyes carinatus* *Pycnophyes communis* *Pycnophyes cf. ponticus*
		3	42°29,226´N 3°08,621'E	25	Fine sand with dentallium	*Pycnophyes carinatus* *Pycnophyes communis* *Pycnophyes cf. ponticus*

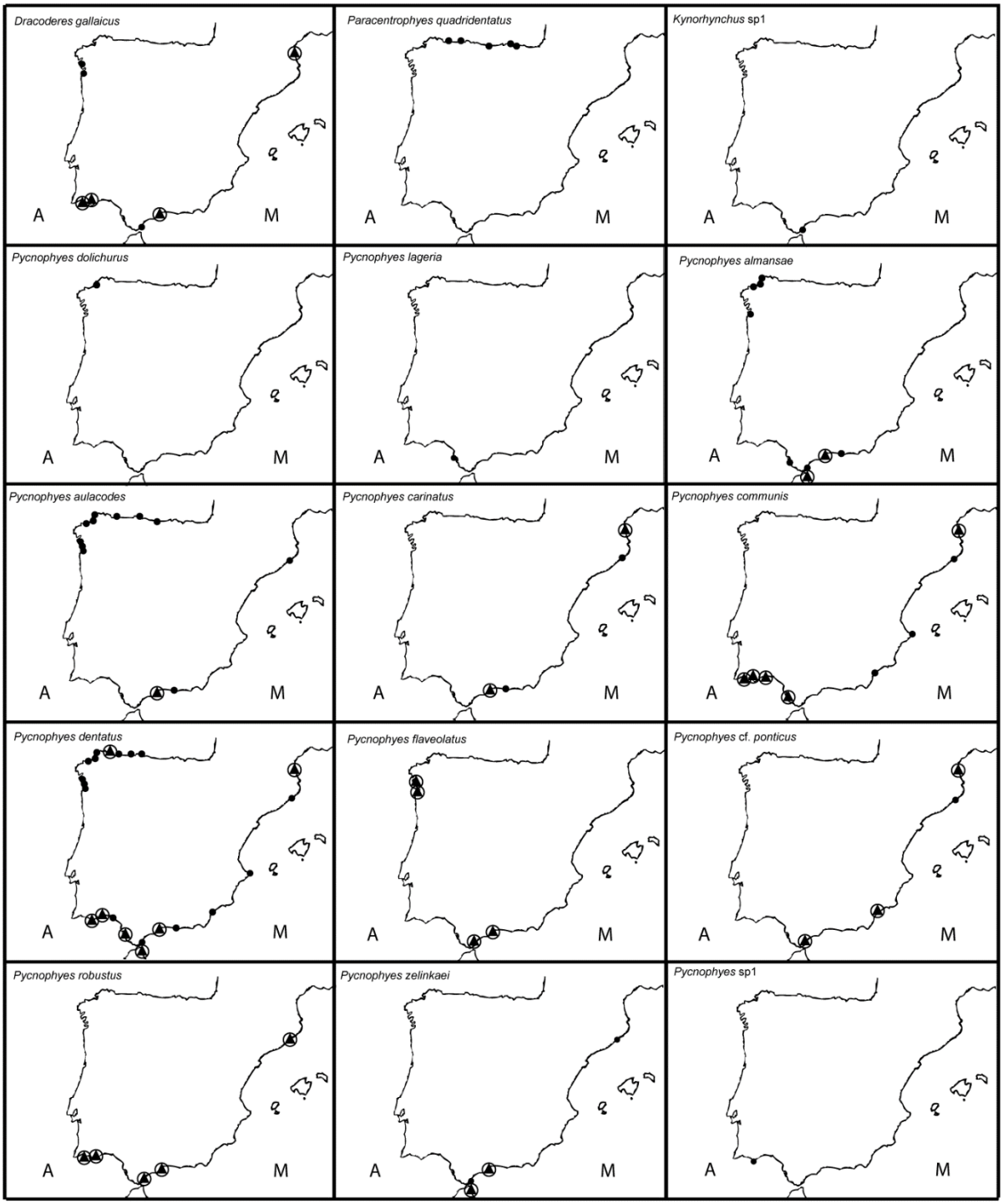


Figure 24. Maps showing the distribution of allomalorhagid species along the Iberian coastline. Black circlets refer to data included in Sánchez et al. (2012), circlets with a triangle refer to new data. Abbreviations: A, Atlantic Sea; M, Mediterranean Sea.

Naples, Italy: A total of five species of allomalorhagids belonging to two families were found. All these species were previously reported by Zelinka (1928).

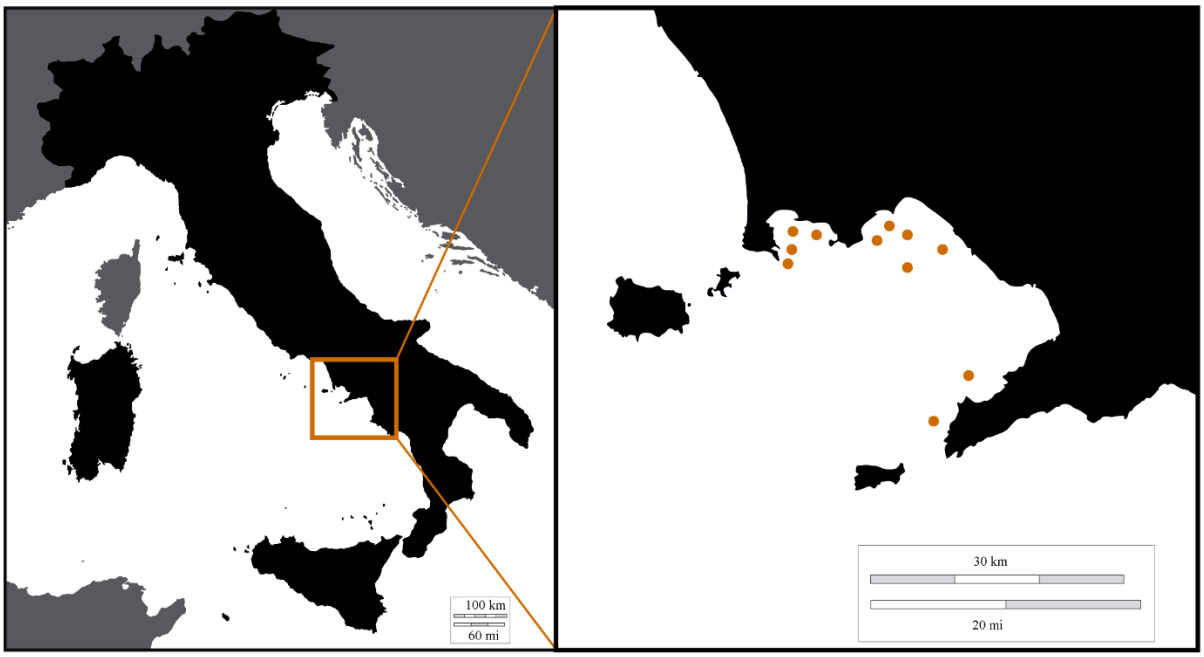


Figure 25. Map showing collecting areas and localities (close-up in the insets) yielding kinorhynchs along the Bay of Naples (Italy).

Table 6. Collecting data and Allomalorhagida species from the Bay of Naples. Collectors: NS, FP, MH.

Locality	Date	Sample	Coordinates	Depth	Sediment	Species
Bay of Naples	04.09.2013	1	40°46,72'N 14° 06,79'E	37-53	Muddy sand	*Pycnophyes cf. ponticus* *Pycnophyes robustus* *Pycnophyes zelinkaei*
		2	40°47,39'N 14°05,71'E	30-52	Mud	*Pycnophyes flaveolatus* *Pycnophyes robustus*
		3	40°48,37'N 14°05,12'E	30-40	Mud	*Pycnphyes flaveolatus* *Pycnophyes cf. ponticus* *Pycnophyes rugosus*
		4	40° 48,49'N 14° 07,85'E	40-50	Sandy mud	*Pycnophyes flaveolatus* *Pycnophyes cf. ponticus*
	05.09.2013	1	40° 48.98'N 14° 13.12'E	29-30	Mud	*Pycnophyes flaveolatus* *Pycnophyes cf. ponticus* *Pycnophyes rugosus*
		3	40° 49,51'N 14°14,28'E	24-30	Sandy mud	*Pycnophyes flaveolatus* *Pycnophyes cf. ponticus* *Pycnophyes robustus* *Pycnophyes rugosus* *Pycnophyes zelinkaei*
		4	40° 48,77'N 14° 15,36'E	22-26	Muddy sand	*Pycnophyes zelinkaei*

		5	40° 47,16’N 14° 14,91’E	98-99	Mud	*Paracentrophyes quadridentatus*
	06.09. 2013	1	40° 36,05’N 14° 19,04’E	56-65	Muddy sand	*Pycnophyes robustus* *Pycnophyes zelinkaei*
		2	40° 38,68’N 14° 21,78’E	99-100	Mud	*Paracentrophyes quadridentatus* *Pycnophyes zelinkaei*
		5	40° 47,83’N 14° 19,55’E	58-63	Mud	*Pycnophyes cf. ponticus*

Norway, Espegrend: A total of five species of allomalorhagids belonging to two families were found, all of them new citations.

Greenland: Three species of Pycnophyidae were recorded, two of them new for the area.

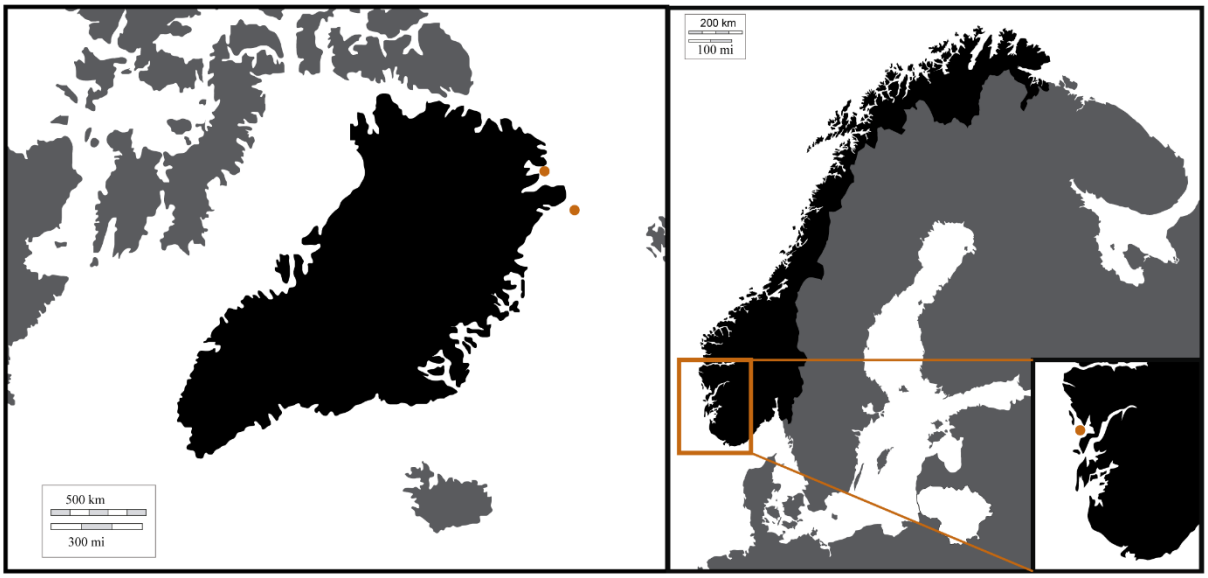


Figure 26. Map showing collecting areas and localities (close-up in the insets) yielding kinorhynchs in Greenland and Norway.

Table 7. Collecting data and Allomalorhagida species from Greenland and Norway. Collector of Greenland samples, RMK (Reinhardt Møbjerg Kristensen); collectors of the Espegrend samples: NS, FP, MH, MVS (Martin V. Sørensen), HY (Hiroshi Yamasaki), SS (Stephen Sanders).

Locality	Date	Sample	Coordinates	Depth	Sediment	Species
North East Water Polynia	02.08.1992	36/SC23	-	-	-	*Pycnophyes greenlandicus*
	02.08.1992	63/SC56	-	-	-	*Pycnophyes* sp6
	02.08.1992	43/SC37	80°19'28"N 9° 28'04"W	310	Mud	*Pycnophyes* cf. *arctous* *Pycnophyes* sp6
Independence Fjord	06.08.1995	33	82°11'N 30° 24'W	30	Muddy, brown clay	*Pycnophyes* cf. *arctous* *Pycnophyes greenlandicus*
Espegrend	22.08.2012	1	60°15,968'N 5° 12,088'E	99	Mud with gravel and rocks	*Paracentrophyes quadridentatus* *Pycnophyes aulacodes* *Pycnophyes zelinkaei* *Pycnophyes* sp7 *Pycnophyes* sp8

East coast of USA: Maine; Woods Hole, Massachusetts; Fort pierce, Florida. A total of three species of allomalorhagids belonging to one family were found. *Pycnophyes norenburgi* was described as new species in Herranz et al. (2014b).

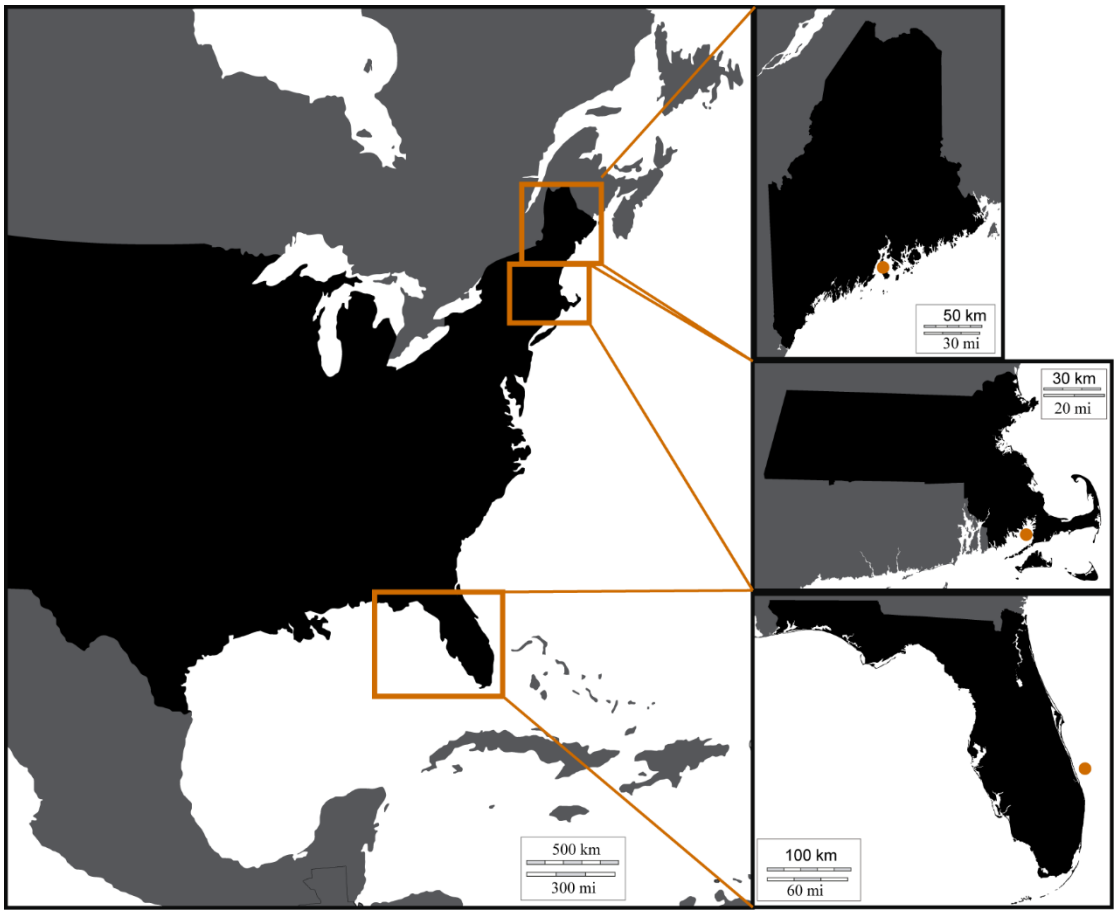


Figure 27. Map showing collecting areas and localities (close-up in the insets) yielding allomalorhagid species along the West Coast of USA.

Table 8. Collecting data and Allomalorhagida species collected along the East coast of USA. Collectors FP, MH, MVS.

Locality	Date	Sample	Coordinates	Depth	Sediment	Species
Maine	-	-	-	-	-	*Kinorhynchus mainensis*
Massachusetts	10.08.2011	1	41°34'54"N 70° 41'24"W	15	Mud	*Pycnophyes frequens* *Kinorhynchus mainensis*
Florida	03.08.2011	20 miles	27°30,84'N 79° 54,86'W	152	Mud	*Pycnophyes norenburgi*

Panama: Bocas del Toro (Caribbean Sea), Naos (Pacific Ocean). A total of seven species of allomalorhagids belonging to three families were found.

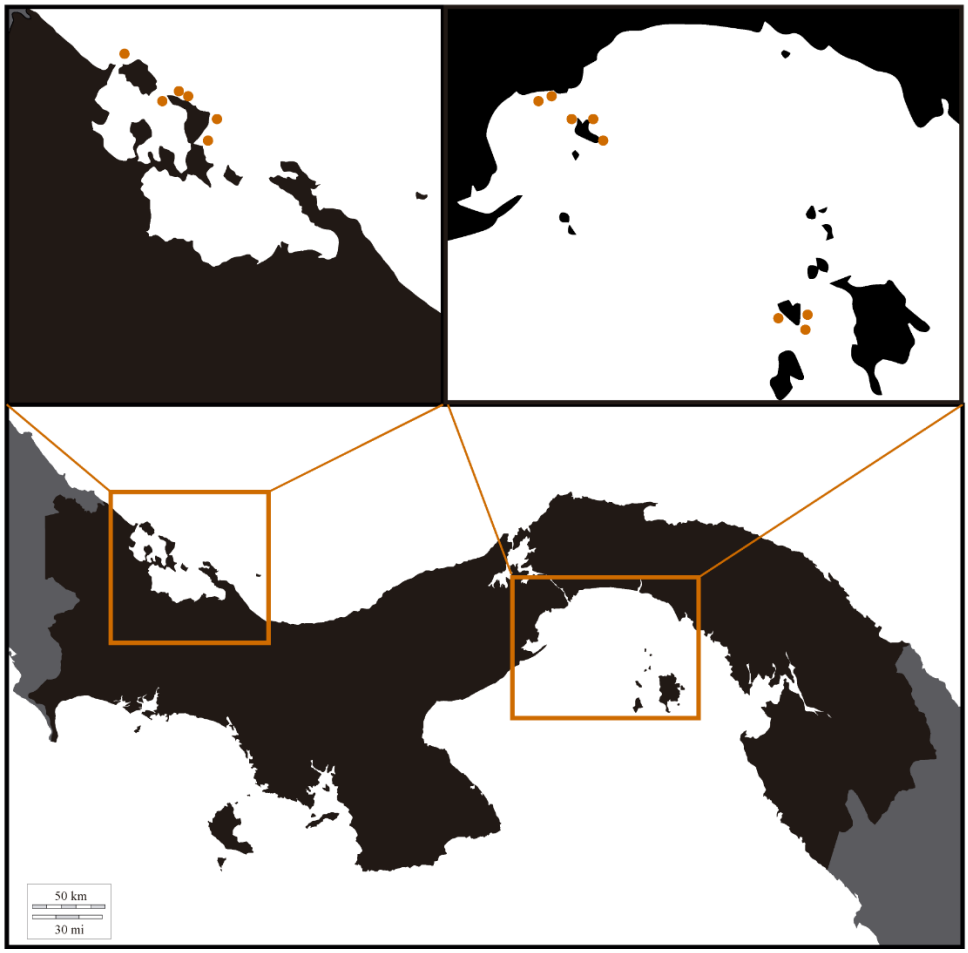


Figure 28. Map showing collecting areas and localities (close-up in the insets) yielding kinorhynchs in Panama.

Table 9. Collecting data and Allomalorhagida species collected at both side of Panama, at the Atlantic and the Pacific coast. Collectors FP, AM (Alejandro Martínez).

Locality	Date	Sample	Coordinates	Depth	Sediment	Species
Bocas del Toro	08.06.2010	BRS 102	9°20,041'N 82°13,137'W	12	Mud	*Paracentrophyes predictus* *Pycnophyes* sp9 *Pycnophyes* sp10
	10.06.2010	BRS 104	9°21,016'N 82°10,335'W	14	Mud	*Paracentrophyes predictus*
	13.06.2010	BRS 106	9° 17,175'N 82° 5,065'W	N/A	N/A	*Paracentrophyes predictus* *Pycnophyes* sp9 *Kinorhynchus* sp2
		BRS 107	9° 15,126'N 82° 7,717'W	N/A	N/A	*Paracentrophyes predictus* *Kinorhynchus* sp3

	17.06.2011	BRS 115	9° 21,039'N 82°10,345'W	N/A	N/A	*Kinorhynchus* sp2 *Kinorhynchus* sp3
Naos	06.12. 2011	7	8° 53,449'N 79° 35,719'W	Intertidal	Mud	*Kinorhynchus* sp4 *Kinorhynchus* sp5
	09.12.2011	18	8° 48,216'N 79° 33,245'W	9	Fine sand	*Kinorhynchus* sp4
		19	8° 46,938'N 79° 32,217'W	8	Medium sand	*Kinorhynchus* sp4 *Kinorhynchus* sp5

Singapore: One allomalorhagid species belonging to the family Pycnophyidae was found.

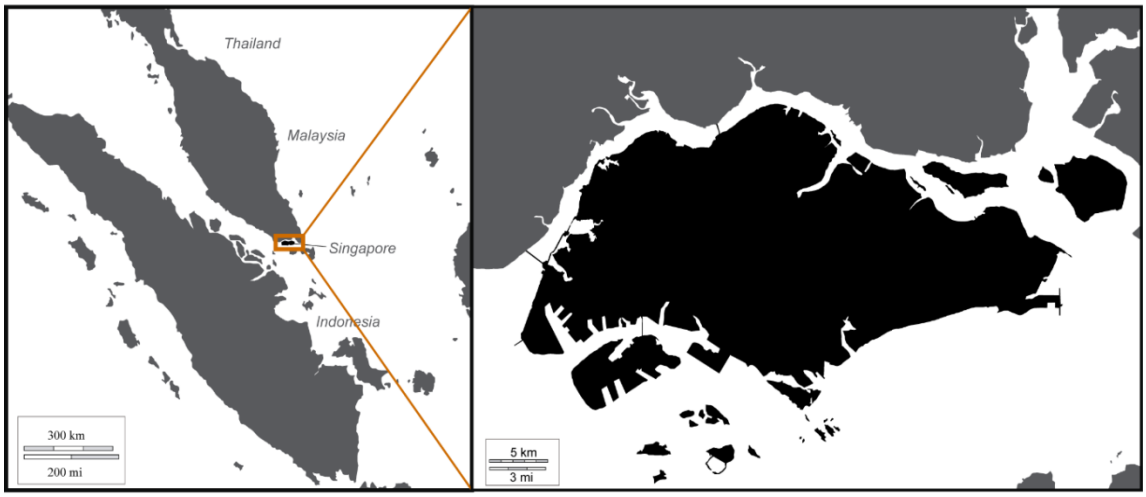


Figure 29. Map showing collecting areas and localities (close-up in the insets) yielding kinorhynchs in Singapore.

Table 30. Map showing collecting areas and localities (close-up in the insets) yielding allomalorhagid species at Singapore. Collector RN.

Locality	Date	Sample	Coordinates	Depth	Sediment	Species
Singapore	16.05.2014	SI-05	01°15'35"N 103° 56'41"E	52	-	*P.*sp11

4

DISCUSSION

After the extensive researches provided by Zelinka, Higgins and Adrianov (Zelinka, 1928; Higgins, 1983; Adrianov and Malakhov, 1999a) the present Thesis represents the largest contribution to the knowledge of the Allomalorhagida. It compiles the description of eleven species, ten of them belonging to the Pycnophyidae and one to the Neocentrophyidae, and sheds light into the internal relationships of Pycnophyidae for the first time.

The driving thread that integrates the different researches compiled in this Thesis derives directly from the Scientific Method and the research progress that follows a never ending thread, where every finding or result leads to one or several new ones. New findings of kinorhynch specimens gave rise to both species descriptions and biogeographical records. These descriptions revealed new or striking features that needed evaluation for phylogenetic purposes, for biogeographical approaches or induced the application of new research techniques. Thus the body of scientific information presented herein derives directly from a research line well stablished by our research team, constitutes a substantial step on the knowledge of the phylum and furthermore, opens new windows to future researches in the field.

A panoramic, integrative view on the main topics to be discussed follows, although detailed treatments can be found at the respective “Discussion” sections of the papers presented (Chapters I, II, III).

4.1. DIVERSITY OF ALLOMALORHAGIDA

One major problem that came up at the beginning of the allomalorhagid studies was related to the terminology associated to specific morphological characters and their position along the trunk. The terminology associated to the cuticular structures was confuse and inconsistent throughout the literature (Zelinka, 1928; Higgins, 1964, 1965, 1969a, 1983; Adrianov and Malakhov, 1999a; Neuhaus, 1993, 1995, 2013; Lemburg, 2002). In order to perform accurate and homogeneous taxonomical descriptions, cuticular characters should be able to be plotted unequivocally on a map following well stablished standards.

The positions of cuticular structures in the trunk, together with their associated terminology, was previously established on a transverse section of a specimen mounted dorsoventrally for light microscopy and was summarized in three series: dorsal, lateral and ventral (Pardos et al., 1998). Unfortunately, these positions were developed on the cyclorhagid trunk shape. Cyclorhagids and most of the allomalorhagids differ considerably in their trunk shape in cross section, triangular for allomalorhagids and rounded or heart-shaped for cyclorhagids. Consequently, the application of the same terminology for both groups results, at least, extremely difficult. Different trunk shape means that the term “lateral” refers to a curved outline covering a broad area in cyclorhagids but just a narrow, sharp angle in most allomalorhagids (the older homalorhagids: Pycnophyidae and Neocentrophyidae, hereafter “traditional homalorhagids”). Hence, the number of positions in the lateral series is potentially higher in cyclorhagids than in the traditional homalorhagids. For instance, no species of Pycnophyidae or Neocentrophyidae has ever been described having cuticular structures in sublateral or accesory positions. By contrast, cuticular structures are commonly reported in such positions for cyclorhagids. So, the single reason for which these two positions have not been reported neither in Pycnophyidae nor Neocentrophyidae is because its lateral area is highly reduced and, subsequently, some positions are just missing. So established, this new terminology agrees with that of cyclorhagids (Pardos et al., 1998), just differing in the lack of the sublateral and accesory positions in the lateral series. The new position terminology should not disturb phylogenetic analyses, basically because there is no way to compare the position of a given cuticular structure between two groups (Cyclorhagida and Allomalorhagida) if this position is lacking in one of them. What could be compared at high taxonomic levels is the presence/absence of the character at the whole “lateral series” (see Sánchez et al., 2011 in Chapter I).

Accordingly, many specific morphological characters were reevaluated with refined definitions in the different families (Sánchez et al., 2011, 2014a in Chapters I, II). Examples are the normalization of the term “setae”, which bring together literature descriptions of “tubes”, “sensory

hairs" or "bristles" or the precise definition of middorsal cuticular specializations, now categorized into "spines", "spinose processes", "processes" and "elevations". An exhaustive list of definitions can be found in the Appendix I.

In the end, these two terminological problems, that is, the accurate definition of morphological characters in allomalorhagids and their precise location along the trunk, could be contributing to the little attention paid historically to the group. If we revise the taxonomic works looking back from the 80' to the beginning of this Thesis, it is remarkable that cyclorhagid descriptions highly outnumber the allomalorhagids ones (Adrianov and Malakhov, 1999a; Pardos et al., 1998; GªOrdóñez et al., 2008; Song and Chang, 2001; Lemburg, 2002; Neuhaus and Blasche, 2006; Sørensen, 2006, 2007, 2008; Sørensen et al., 2005, 2007; Sørensen and Rho, 2009; Neuhaus, 2013; Neuhaus et al., 2013; Neuhaus and Sørensen, 2012). This different research effort applied to cyclorhagids vs. allomalorhagids is also reflected in the number of genera described recently. In the last ten years, six out of eight of the newly described genera were cyclorhagids (Neuhaus and Blasche, 2006; Sørensen et al., 2007; Sørensen, 2008; Sørensen and Rho, 2009; Sørensen and Thormar, 2010; Herranz et al., 2012), one, *Franciscideres*, was accommodated into the allomalorhagids only according to phylogenetic data, even though its morphology resembles cyclorhagids (Dal Zotto et al., 2013; Sørensen et al., in press), and only one, *Mixtophyes,* is clearly aligned with allomalorhagids. Therefore, we can infer that the scarce discoveries of new allomalorhagid genera may be consequence of a lesser strong research effort and not necessarily reflecting a real lower diversity at the genus level.

Since the new allomalorhagid terminology came out (Sánchez et al., 2011 in Chapter I), several authors joined up in the studies of the group, as H. Yamasaki, M. V. Sørensen and M. Maiorova (Sørensen et al., 2010a; Yamasaki et al., 2012; Adrianov and Maiorova, 2014). Most of the authors and researchers on the phylum agreed with the new terminology and positions, and subsequently adopted it in their publications. Notably, this new system was not followed by Neuhaus (2013). In some sense, an unexpected consequence of the new terminology was to encourage new studies and authors into the group.

Eleven new species of Allomalorhagida have been described during the developing process of the present Thesis. One of them, *Mixtophyes abyssalis*, belongs to the Neocentrophyidae and constitutes the first description of an allomalorhagid genus since more than 30 years from present (Higgins, 1983; Sánchez et al., 2014a in Chapter II). The remaining ten new species described herein were accommodated into the Pycnophyidae (Sánchez et al., 2011, 2013, 2014b, 2014c in Chapter I). It is noteworthy that four of them are the first allomalorhagid species described from the Iberian

Peninsula (Sánchez et al., 2011, 2014c in Chapter I). The ten new species together with *Pycnophyes norenburgi* (described by our research team, Herranz et al., 2014b but not included in the present Thesis and *P.* sp11, currently under revision) increased by a 20% the total number of valid species of Pycnophyidae worldwide, bringing it from 45 up to 56 species.

4.1.1. The family Neocentrophyidae

Implications for Systematics

The re-observation of the single two species of ***Neocentrophyes*** revealed novel and relevant information about their morphology and hence, emended diagnoses for the genus and family levels were required and performed (Sánchez et al., 2014a in Chapter II). The most relevant morphological information was the arrangement of the sternal plate of the terminal trunk segment, not differentiated into two sternal plates as originally reported (Higgins, 1969) but as a single, undivided plate (Sánchez et al., 2014a, Chapter II). This observation was of major interest since the presence of a single sternal plate on the terminal segment had been reported for the remaining known species of the family (Higgins, 1983; Sørensen et al., 2010a; Sánchez et al., 2014a in Chapter II) and therefore now this becomes a synapomorphic character shared by all species in the family. In addition, the observation of the type material of both species allowed us to discriminate and correct several morphological shortcomings found in the original descriptions, regarding the position of the penile spines, the presence of a middorsal, basally articulating spine on segment 11 in females, a pair of articulated bulbous protrusions with thick cuticle at the same position as the lateral terminal spines and the absence of ventrolateral setae on segment 4. This last observation was of major relevance since the two species of *Neocentrophyes* described so far are currently distinguished only by characters related to sexual dimorphism and therefore it is even more likely that both species could be actually female and male of the same species, a circumstance already pointed out by Higgins (1983) (Sánchez et al., 2014a in Chapter II).

The discovery of ***Mixtophyes*** means the first description of an allomalorhagid genus since more than 30 years ago (Higgins, 1983; Sánchez et al., 2014a in Chapter II). The morphology of *Mixtophyes* perfectly fits with those of the old homalorhagids, that is, *Pycnophyes*, *Kinorhynchus*, *Paracentrophyes* and *Neocentrophyes*. The new genus was clearly accommodated into the Neocentrophyidae due to its composition of the sternal plate on segments 1 and 11.

The phylogenetic relevance of traditional taxonomic characters

Both the organization of segment 1 and the presence of lateral terminal spines have always played a major role into the systematics of kinorhynchs. Especially, the latter has been a key character for allomalorhagids. After the novel information reported by Sánchez et al. (2014a) (Chapter II), the **sternal plate composition of the segment 11** may also be considered as a relevant character for the systematics of Neocentrophyidae since all species show a single sternal plate on this segment. However, such arrangement seems to be homoplastic into the phylum (Sánchez et al., 2014a in Chapter II), and may vary even at intrageneric levels (Neuhaus and Blasche, 2006; Sørensen et al., 2013). This hypothesis on the homoplasy has been corroborated recently by combined morphological and molecular phylogenetic analyses (Sørensen et al., in press).

If we back to the other two main features of the family, that is, the lateral terminal spines and the composition of the segment 1, *Mixtophyes* shows a mixture of the diagnostic characters of both *Paracentrophyes* and *Neocentrophyes* (Higgins, 1969, 1983; Sánchez et al., 2014a in Capter II; Sørensen et al., 2010a).

According to Sánchez et al. (2014a) (Chapter II), the presence of **lateral terminal spines** may be the plesiomorphic condition for kinorhynchs and, consequently, their loss is a homoplastic character that evolved convergently several times into Allomalorhagida. Thus, the relationships based on this character would be disregarded. The hypothesis has been recently confirmed through combined molecular and morphological phylogenetic analyses (Sørensen et al., in press) and hence, the presence of this trait cannot be used to stablish a phylogenetic relationship of *Mixtophyes* and *Paracentrophyes*.

As for the **composition of the segment 1**, most experts on kinorhynchs considered the partially divided sternal plate in *Paracentrophyes* as an intermediate condition between a single, undivided sternal plate (*Neocentrophyes* and *Mixtophyes*), and a fully divided one (*Pycnophyes* and *Kinorhynchus*). They disagreed, however, about the polarity of this character transformation. Following basically this idea, the last hypothesis about the transformation series of segment 1 was proposed by Sánchez et al. (2014c) (Chapter II). The authors proposed a situation in which the first trunk segment formed by a closed ring evolved into one tergal and one undivided sternal plates, as in *Neocentrophyes* and *Mixtophyes*. Then, the sternal plate became partially divided, as in *Paracentrophyes*, and finally the sternal plate evolved into totally divided, as in *Pycnophyes* and *Kinorhynchus*. The opposite direction of the evolutionary scenario seemed more unlikely after the topologies obtained in the molecular phylogenetic analyses performed by Yamasaki et al. (2013) and Dal Zotto et al. (2014). From this point of view, the presence of a single, undivided sternal plate on

segment 1 would represent the plesiomorphic condition for a clade formed by the old homalorhagid genera. Hence, its presence in both *Neocentrophyes* and *Mixtophyes* cannot be used as an argument to consider the two genera as closest relatives.

Therefore, and after considering the arrangement on segment 11 and the presence of lateral terminal spines as characters without valuable phylogenetic information, the plate configuration of segment 1 suggested an evolutionary scenario with *Paracentrophyes* as the sister taxon to *Pycnophyes* and *Kinorhynchus*, with *Neocentrophyes* and *Mixtophyes* branching off firstly (Sánchez et al., 2014a in Chapter II). Even though this hypothesis was not derived from phylogenetic studies the monophyly of Neocentrophyidae revealed questionable.

Soon thereafter this hypothesis came out, phylogenetic analyses including molecular and morphological data confirmed the first evolutionary step of this character transformation, from a ring-like as starting point towards one tergal and one sternal plate (Sørensen et al., in press; Sánchez et al., in press in Chapter III). The ring-like plesiomorphic condition was retained in Dracoderidae, now within the Allomalorhagida. Unfortunately, the character reconstruction for segment 1 was recovered equivocally for Franciscideridae and Neocentrophyidae. Neither its plesiomorphic condition nor its transformation within both families could be confirmed in the phylogenetic analyses (Sørensen et al., in press). Hence, the subsequent steps of the suggested character transformation, from a single undivided sternal plate (*Neocentrophyes* and *Mixtophyes*) towards a partially divided one (*Paracentrophyes*) and lastly into a totally divided sternal plate (*Pycnophyes* and *Kinorhynchus*) could not be confirmed (Sørensen et al., in press). It can be concluded from the same analyses that the suggested relationship within the Allomalorhagida referred to above as proposed in Sanchez et al. (2014a) should be rejected. In fact, in the topology obtained (see Fig. 16 in *Introduction*), Pycnophyidae appeared as the sister taxon of a large clade including Franciscideridae and Neocentrophyidae, with *Franciscideres* and *Paracentrophyes* branching off firstly (Sørensen et al., in press).

The traditional idea about a partially divided sternal plate, as a real intermediate stage between the undivided and the totally divided sternal plate, may be at least somehow questionable, according to the topologies yielded in Sørensen et al. (in press) and Sánchez et al. (in press) (Chapter III). The topology of the first analysis showed Franciscideridae and Neocentrophyidae as sister taxa with Pycnophyidae branching off (Sørensen et al., in press), and the second one showed Pycnophyidae as sister taxon to Neocentrophyidae (Sánchez et al., in press, in Chapter III). Therefore, and even though the character reconstruction was equivocal, it is not required under these topologies to pass through the “intermediate step” to go from one stage to the other, that is, from

undivided to totally divided or *vice versa*. The new hypothesis would be that the sternal plate of segment 1 evolved from the plesiomorphic ring-like into two independent paths, one for Francisciderida + Neocentrophyidae (ring-like and sternal plate partially divided or without any division, respectively) and one for Pycnophyidae (sternal plate fully divided). Conclusively, the role of the partially divided sternal plate as an evolutionary intermediate state would leave rejected. It could happen that too much emphasis has been put on the intermediate stage of the partially divided sternal plate of *Paracentrophyes*, conditioning to some extent the results of the phylogenetic analysis through the polarity of character transformations.

4.1.2. The family Pycnophyidae

Implications for Systematics: the family grows up…

Pycnophyidae is the second largest family within the phylum, accommodating one-third of the described species (Sørensen, 2013). Hence, it is not surprising that most of the new species or new citations compiled into the present Thesis belong to this family. Ten new species of Pycnophyidae included herein were described from the Iberian Peninsula, the Korean Peninsula and the Guinea Basin: *Pycnophyes aulacodes*, *Pycnophyes dolichurus*, *Pycnophyes almansae*, *Pycnophyes lageria*, *Pycnophyes pardosi*, *Pycnophyes chalgap*, *Pycnophyes cristatus*, *Pycnophyes smaug*, *Pycnophyes nubilis* and *Pycnophyes farinellii* (Sánchez et al, 2011, 2012, 2013, 2014b, c; see Chapter I and Table 4 in *Material and Methods* for the new genera assigned). Therefore, and as noted above, the present study implies an increase on the diversity of Pycnophyidae around 20%.

…and growing pains: realizing uncommon characters within the family

Four out of the referred ten new species are easily distinguished by any of two uncommon features within the family. *Pycnophyes cristatus* and *Pycnophyes nubilis* share the presence of keel-shaped middorsal processes on segments 1-10 (Sánchez et al., 2013, 2014c, in Chapter I), a feature also present in another 11 species of Pycnophyidae (Zelinka, 1928; Lang, 1949, 1953; Higgins and Kristensen, 1988; Higgins, 1991, 1983; Adrianov and Malakhov, 1999a; Adrianov and Maiorova, 2014; Sánchez personal observation for one species).

On the other hand, *Pycnophyes dolichurus* and *Pycnophyes farinellii* are characterized by the lack of male-specific tubes on segment 2 (Sánchez et al., 2011, 2014c; in Chapter I). The presence of these tubes in males is a widespread character among the species of both *Kinorhynchus* and *Pycnophyes*, whereas the remaining allomalorhagids lack them (Neuhaus, 2013). Only seven additional *Pycnophyes* species lack such male-specific tubes (Lang, 1953; Higgins, 1966a, 1983;

Sánchez, personal observation for two species). Moreover, their absence seems to be linked to the presence of relatively long lateral terminal spines in both sexes (values equal or higher than 30% measured as lateral terminal spines/total trunk length). This observation led to speculate about a possible closer relationship between these nine species (see Sánchez et al., 2014c in Chapter I).

In summary, both features, the middorsal keels and the absence of male tubes, suggested some hypothetical close relationships that required phylogenetic analyses of the whole Pycnophyidae in order to test the monophyly of these two potential species groups.

4.2. PHYLOGENY UNDER TOTAL EVIDENCE ANALYSIS: RE-ARRANGING THE CHAOS BASED ON NATURAL GROUPS

The idea about performing a phylogeny focused on the Pycnophyidae was motivated by several reasons. Firstly, to test the hypothesis recently proposed by Sánchez et al. (2014c) (Chapter I) suggesting that the pool of *Pycnophyes* species without ventral tubes in males could be closely related. Secondly and following the same argument, to verify additional unpublished hypothesis on the relationships between the species with keel-like middorsal processes, and to trace the evolution of the middorsal structures if this feature has phylogenetic relevance. Third, to perform a throughout revision on the systematics of the whole family with a sound basis on phylogenetic relationships.

The monophyly of Pycnophyidae was fully supported in all previous phylogenetic analyses of the phylum (either based on molecular data or using combined molecular and morphological data) (Dal Zotto et al., 2013; Yamasaki et al., 2013; Sørensen et al., in press). However, all analyses failed to recover the genera *Pycnophyes* and *Kinorhynchus* as monophyletic groups. Similar topologies regarding the internal relationships of Pycnophyidae were obtained, with *Pycnophyes* and *Kinorhynchus* as paraphyletic and polyphyletic, respectively. This premise on the paraphyly of *Pycnophyes* was previously suggested by several authors on the fact that *Kinorhynchus* is exclusively distinguished from *Pycnophyes* by the absence of lateral terminal spines (Higgins, 1962; Brown, 1985; Neuhaus, 1993; Lemburg, 2002).

Therefore, we considered a major goal at that point to test hypothesis about the internal relationships of *Pycnophyes* with the available information to date. Subsequently and, if confirmed, a new systematic arrangement inferred from phylogenetic relationships would be required for the family.

4.2.1. Overcoming the sampling problems: the landing of morphology

Pycnophyidae is at present the second largest family of Kinorhyncha, accommodating one-third of the total described species. It includes the second and third most diverse genera of the phylum, that is, *Pycnophyes* with 56 species and *Kinorhynchus* with 19 (Neuhaus, 2013; Sørensen, 2013; Sánchez et al., 2014b). Despite the increased sampling effort by various researchers during the last decade, the number of sequenced species of Pycnophyidae for molecular studies is still very low, a situation mostly derived from identification problems and lack of skilled taxonomists. This is noticeable from the most comprehensive analyses of kinorhynch interrelationships carried out so far, which included eight species of *Pycnophyes* and only two species of *Kinorhynchus* vs. around 40 species of cyclorhagids (Sørensen et al., in press). The samples bias in the number of sequenced *Pycnophyes* species in contrast to the low number of those of *Kinorhynchus* could have caused the

nested appearance of the latter amongst the species of the former. The perfect situation in order to test the monophyly of both genera would be to obtain as much as possible molecular data from the species of the family. However, for an elusive group like Kinorhyncha this idea is not as simple as it may sound: many species were always collected in low abundance and most of them have a restricted distribution area, or are exclusively known from a single sample at the Polar Regions or the deep-sea (Adrianov and Malakhov, 1999a; Sánchez et al., 2012). This makes resampling very difficult and specimens hardly available for molecular analyses. Hence, for now, a taxon sampling covering most of the pycnophyids is not realistic. Therefore and all in all, the single way to overcome the lack of information for many species is through the inclusion of the morphological characters with potential phylogenetic significance for the family. This has the additional bonus of a more holistic approach to the problem.

The inclusion of the morphological partition resulted in lower nodal supports and polytomies but at the same time has a lot of advantages. Firstly, as we previously stressed, the use of morphology enabled us to incorporate all the described species into the analysis. Secondly, it lets to find apomorphies to define and support the clades, allowing new forthcoming species to be assigned to a specific genus easily. Moreover, we may test morphological hypothesis on the body plan evolution proposed in previous morphological studies through morphological character reconstructions. Moreover, the tracing allow us to discriminate which morphological characters have phylogenetic relevance and which are homoplastic. In this way, we may understand the evolution of body plan within the group. Ultimately, the final interest of the zoologists who study phylogeny is to know how the animals evolve, how they adapt to the environment and how these adaptations are reflected in their phenotype.

Likewise, and applied to our particular study, the total evidence analyses yielded several large clades of taxa without molecular data of any species. Therefore the morphology revealed these clades that would keep hidden in other way. In addition, the total evidence analyses helped to solve the position of the rogue taxa in the molecular and combined analyses. See, for example, the cases of *Cristaphyes yushini* and *Pycnophyes zelinkaei* in Chapter III. The rogue behavior of these species was likely related to their divergent morphology and insufficient taxon sampling. Probably, the discovery of new taxa might provide further information allowing the proper placement of these and additional problematic species (see discussion on the emended genus *Pycnophyes* in Chapter III).

Lastly, morphology compensates the geographical bias of our molecular dataset, just consequence of the sampling effort. This is a common problem in many phylogenetic analyses and general taxonomic studies that might confound the actual biogeographic patterns in certain groups (Curini-Galletti et al., 2012). In our case, inclusion of all described species revealed an entire clade

dominated by deep-sea and Artic species. Using the molecular data set alone, this clade would be represented by a single species.

Traditionally, morphology had a key role in animal phylogeny. Since the end of the XX century, with the arrival of molecular techniques, some authors considered morphology as outdated or "old-fashioned" science. This thought was even more spread when the use of molecular approaches in phylogeny reached an exponential development, yielding a vast amount of data in short times and with low costs. However, morphology should not be pushed into the background, basically because the interpretation of the gene expression cannot work without a deep knowledge of morphology. Or in other words, the expression of morphological phenotypes is the result of underlying cellular genes. Therefore, and other than basic science, one of the major modern contributions of morphology to phylogeny is to assess and make sense to the trees rather than just building trees. In fact, the consistence of the phylogenetic tree would be greater as much as both molecular and morphological data are congruent. Such integrative view is receiving increasing support by the scientific community (Giribet, in press.; Wanninger, 2015) and is being applied congruently to a variety of groups (see, for example, Martinez et al., 2014; Di Domenico, 2014).

4.2.2. Taxonomic implications of the analysis: new classification of Pycnophyidae

The genera *Pycnophyes* and *Kinorhynchus* were not recovered as monophyletic groups, in any of our analyses (Sánchez et al., in press, in Chapter III). The species of *Kinorhynchus* always appeared nested amongst *Pycnophyes,* a fact that has two consequences. First, the suggested paraphyly of *Pycnophyes* was verified by our results; and second, *Kinorhynchus* is polyphyletic and hence is not valid as a taxonomic and phylogenetic unit. *Kinorhynchus* is no longer considered an allomalorhagid genus and consequently synonymized with *Pycnophyes*, this latter retaining the priority and surviving redefined as one of the genera of the family.

The topology yielded by the total evidence analyses allowed us to identify some major clades that we consider well-supported and valid. Pycnophyidae was recovered as divided in nine clades, all of them supported by molecular and morphological apomorphies and hence each one was given a hierarchical category in the taxonomy of the family. The newly erected genera are *Planolimbus, Cristaphyes, Higginsia, Gymnophyes, Setaphyes, Fujuriphyes, Krakenella,* and *Godzilliphyes* (See Chapter III).

The lower nodal support of the clades "*Planolimbus*", "*Higginsia*" and "*Cristaphyes*" are most probably the result of the high amount of missing data because their respective pools of species were

represented only by the morphological partition (except one species in "*Cristaphyes*"). Further studies, hopefully incorporating molecular data for these clades, will be needed to give them stronger support.

4.2.3. The phylogenetic relevance of traditional taxonomic characters, second round!

Losers: Conspicuous characters without phylogenetic relevance within Pycnophyidae

Lateral terminal spines: Traditionally, the differential, diagnostic character to discriminate between *Pycnophyes* and *Kinorhynchus* is the presence of lateral terminal spines in the former, lacking in the latter (Zelinka, 1928; Sheremetevskij, 1974; Higgins, 1983; Adrianov and Malakhov, 199a; Sørensen and Pardos, 2008). However, the re-observation of most *Kinorhynchus* type material revealed novel and relevant information about this feature. The species of *Kinorhynchus* bear articulated bulbous protrusions with thick cuticle, a central canal and a blunt terminal end at the same position as the lateral terminal spines in *Pycnophyes* (see Sánchez et al., 2014a in Chapter II). In fact, these bulbous structures resemble the developmental stages of the lateral terminal spines in *Pycnophyes*, *Paracentrophyes* and *Mixtophyes* (Neuhaus, 1993; Neuhaus, 1995; Lemburg, 2002; Sánchez et al., 2014a). This statement suggested that the alleged absence of lateral terminal spines in *Kinorhynchus* actually represents a rudimental stage of the structure, which has evolved convergently.

Neither our topologies nor the character tracing supported the monophyly of *Kinorhynchus*, so the absence –or better, the rudimental stage- of lateral terminal spines is a character state without phylogenetic relevance. The character tracing in the topologies yielded by the total evidence analyses showed a high homoplasy for the lateral terminal spines, with several losses within Pycnophyidae (see Sánchez et al., in press, in Chapter III).

Male-specific tubes: Sánchez et al. (2014c) suggested two phylogenetic hypothesis regarding the large ventromedial tubes present in males of most *Pycnophyes* species. The first one addressed that the presence of these tubes was autapomorphic for Pycnophyidae, which has been corroborated by Sørensen et al. (in press) and Sánchez et al. (in press) (Chapter III). The second one pointed out a potentially monophyletic group gathering *Pycnophyes* species with males lacking the ventral tubes. Such species additionally share another uncommon feature within the family, the presence of long lateral terminal spines (lateral terminal spines/total trunk length > 30%). Neither the topologies yielded by the total evidence analyses nor the character tracing supported this hypothesis. Contrarily, these showed several independent losses of tubes in males and non-related gain of large lateral terminal spines in the family (see Sánchez et al., in press, in Chapter III).

Winners: Characters with phylogenetic relevance within Pycnophyidae

The presence of another noticeable feature in some species of Pycnophyidae led us to think on a possible relationship between the species having keel-like middorsal processes. In order to test this hypothesis we went a step further, including in the analysis all the character states that the middorsal structure specializations may present along the phylum: spines, middorsal spinose processes, middorsal processes, middorsal elevations. This way, if the middorsal structure specialization has phylogenetic relevance we could trace the complete evolution of the character throughout the family and, at the same time, contribute to test the initial hypothesis on a potential group of species bearing keel-like middorsal processes.

The character tracing suggested the homology of the different middorsal specializations, with a transformation series from the middorsal spines (plesiomorphic condition for Allomalorhagida, which can occur on any segment from segment 1 to 11 according to Sørensen et al., in press) into middorsal spinose processes on the anterior segments in Neocentrophyidae and into middorsal elevations on all segments in Pycnophyidae, both as apomorphies for the respective families. Within Pycnophyidae, middorsal elevations were either retained in several genera, lost in *Planolimbus*, or transformed into middorsal processes in *Cristaphyes* (and on the posterior segments of *Higginsia Setaphyes dentatus* and *S. flaveolatus*) (see Sánchez et al., in press, in Chapter III).

In addition, a fully supported clade consisting of the species with conspicuous keel-shaped middorsal processes (apomorphy) was recovered within *Cristaphyes.* This clade was also supported by another unique apomorphy, the presence of a prominent keel on segment 10 that surpass the posterior end of the trunk. Lastly, it is noteworthy that this group of species so well-supported within *Cristaphyes* includes most of the deep-sea species (100-5000 m depth). It should be remarked that only one of the 17 species in the genus has been sequenced (see Figure 5 in Chapter III). Hence, further research incorporating more molecular data on the species of *Cristaphyes* would shed light on the systematics of the genus and its internal groupings of species.

4.3. A NEW WINDOW: THE MICRO-COMPUTED TOMOGRAPHY (MICRO-CT)

The new micro-morphological techniques, as the micro-computed tomography (Micro-CT), the synchrotron X-ray tomography, or even the widely used Confocal Laser Scanning Microscopy (CLSM) take the helm of more classical techniques, as the Transmission Electron Microscopy (TEM) or the Scanning Electron Microscopy (SEM). In our case, the non-invasive Micro-CT techniques applied here for the first time to kinorhynchs, surely will provide novel or updated information about the internal anatomy of the group. The functional interrelationship between organ systems, sometimes of high complexity, can be easily understood thanks to the versatile 3D renderings. And this is even better for the study of controversial taxa, often with very few specimens available or even when taxa are represented just by preserved museum specimens. Subsequently, new supplementary data could be incorporated into the morphological matrices in order to make the total evidence analyses more complete, since just a single observation may change the phylogenetic hypotheses.

The preliminary results presented herein are just the first step in the standardizing process of the micro-CT techniques for animals of tiny sizes. Undoubtedly, further development is needed in order to increase and improve the resolution of the images provided, so details can be discerned clearly.

4.4. GEOGRAPHICAL DISTRIBUTION

Despite the great effort that has been done during recent years in kinorhynch research, the available data can still not provide a complete picture of the extant diversity. The current knowledge of **worldwide kinorhynch biogeography** is more likely a reflection of the sampling bias rather than a reliable kinorhynch distribution (Neuhaus, 2013; Appendix II). Only a few regions of the World, such as the North American East Coast, the European West Coast and the Mediterranean Sea, have been extensively surveyed and therefore can be considered relatively well-investigated. However, even within these areas, the discovery of new species or new citations is still frequent. Our additional data compiled herein is intended mainly to contribute to the huge task of completing the worldwide map of the allomalorhagid distribution. We include new data from several sampling campaigns carried out in many places by our research team alone or in collaboration with other researchers. Surveys were developed in Espegrend (Norway), Greenland (Denmark), Maine, Massachusetts and Florida (USA), Bocas del Toro and Naos (Panama), Singapore, Gulf of Naples (Italy) and several localities along the Iberian coasts. The kinorhynch fauna from the Gulf of Naples was previously studied by Zelinka (1928), and our goal was to resample and compare diversity results after 100 years and also to extend the limits of the sampling area, including new localities. Lastly, we wanted to enlarge and

complement the previous biogeographical study in the **Iberian Peninsula** carried out by Sánchez et al. (2012) providing new reports in additional localities. The gathering of a considerable amount of data along the Iberian Peninsula leaded us to perform a more rigorous ecological study now in progress in order to detect significate preferences of species for sediment types and depth as well as an evaluation on the effectivity of the sampling effort.

Combining the new data with those compiled in Sánchez et al. (2012), the current state of kinorhynch research along the Iberian coasts yielded a total of 31 species and 11 genera from 122 sampling localities (see Figure 23 in Appendix II). Thus, almost half of the currently known genera (11 out of 23) has been reported along the Iberian coasts. A total of 15 allomalorhagid species are present in the whole area, accommodated in four different genera: one species of *Dracoderes*, one *Kinorhynchus*, one *Paracentrophyes*, and 12 *Pycnophyes* (see Fig. 24 in Appendix II) (Sørensen et al., 2010a; Sánchez et al., 2011, 2012, 2014b). Eight of them are new records for the Iberian Peninsula (Appendix II and Sánchez et al., 2012).

Despite the high diversity found in the Iberian Peninsula coasts, it is worth pointing out that most samplings were performed in shallow waters (less than 50 m depth). Therefore, it seems likely that extensive samplings in deeper waters can unveil an even higher diversity, which means that the census of Iberian Kinorhynchs may be still far to be complete. Regarding the obtained and potential data it would be tempting to designate the Iberian Peninsula as a new kinorhynch hot spot. However, this high diversity could just be close to a normal one, resulting by comparison that other areas with lower diversities are just poorly sampled. A similar high diversity has been revealed from sampling campaigns along the East coast of USA (Higgins, 1964a,1964b, 1965, 1977b, 1990; Sørensen, 2007; Sørensen et al., 2007; Herranz and Pardos, 2013; Herranz et al., 2014b) and recently in Korean waters (Sørensen et al., 2010a, 2010b, 2010c, 2010d, 2012a, 2012b, 2013; Lundbye et al., 2011; Sánchez et al., 2013; Thomsen et al., 2013; Altenburger et al., 2015) and the newly explored Gulf of Mexico area (Sørensen and Landers, 2014).

The Iberian Peninsula is an especially interesting area for the study of kinorhynchs because of its singular location between the Atlantic Ocean and the Mediterranean Sea. This provides an ideal situation for biogeographical and ecological studies. According to our data, the biological and geographical frontiers between the Atlantic Ocean and the Mediterranean Sea do not seem to be coincident. Most southern “Mediterranean” localities included in our study such as Málaga, Granada and even Almería are highly influenced by Atlantic waters, which enter into the Mediterranean through a strong surface current. Consequently, this would explain that their kinorhynch fauna is more atlantic rather than mediterranean. The distribution of two species, *Echinoderes* sp.2 and

Pycnophyes almansae (Fig. 24 in Appendix II) could exemplify the Atlantic influence on Mediterranean waters. These species are widely distributed in Atlantic coasts but are only present in the Mediterranean at the mentioned localities. However, the Atlantic influence is mostly superficial and we do not know how it can affect the distribution of kinorhynch populations in deeper waters. The dispersal mechanisms of kinorhynch populations are completely unknown, but surely they are not affected by surface currents in deep waters. This opens a new field for kinorhynch research and, extensively, for meiofaunal communities.

Allomalorhagid species with a wide distribution in both Mediterranean and Atlantic localities are: *Pycnophyes almansae*, *Pycnophyes aulacodes*, *Pycnophyes communis*, *Pycnophyes dentatus, Pycnophyes flaveolatus* and *Pycnophyes zelinkaei.* Their broad distribution through northwest Europe suggests a colonization process from Atlantic to Mediterranean waters. Of them, *Pycnophyes flaveolatus* was described from the Gulf of Trieste and Naples (Zelinka, 1928) but latter recorded in the Baltic Sea (Lang, 1936; Nyholm, 1947c, 1976) suggesting a wide distribution in both Atlantic and Mediterranean areas. A special case is *Pycnophyes communis*, which has been considered as a species with a wide Atlantic and Mediterranean distribution (Zelinka, 1928; Nyholm, 1947c). However, recent reexaminations confirmed that it was often mistaken with *Pycnophyes aulacodes* and wrongly reported from different Atlantic localities (Denmark and Sweden) (see Sánchez et al., 2014c). Therefore, *Pycnophyes communis* seems to have a quite more restricted distribution, but it is still present in Atlantic and Mediterranean localities (Fig. 24 in Appendix II).

Contrarily, we could also find exclusive species from Mediterranean localities with a wide distribution such as *Pycnophyes carinatus* (Zelinka, 1928; Sánchez et al., 2012) recently collected in Malaga and Banyuls (Fig. 24 in Appendix II). Special mention deserves the enlarged distribution of two typically considered Mediterranean species *Pycnophyes* cf. *ponticus* (same as P. sp3 in Sánchez et al., 2012) and *Pycnophyes robustus* (new to the Iberian fauna). Both species were previously collected along the Italian coasts (Zelinka, 1928) and the former one was also reported in the Black Sea (Reinhard, 1881; Bacescu, 1968; Sheremetevskij, 1974) and recently at Blanes (Sánchez et al., 2012). The present report of both species at some localities in the Algeciras Bay extends its known distribution area to what could be considered the Atlantic region. As previously explained, the Algeciras area is highly influenced by Atlantic currents entering the Mediterranean and therefore could be considered Atlantic (see Sánchez et al., 2012). Other species appear to have even more restricted distributions, found in a single or very few localities such as *Kinorhynchus* sp.1, *Pycnophyes lageria* and *Pycnophyes dolichurus* (Fig. 24 in Appendix II). However, future samplings will be

necessary in order to consider them as having restricted distributions or just being the reflection of local findings.

5

CONCLUSIONS

Diversity and taxonomy

- An accurate terminology for the specific morphological characters of Allomalorhagida has been established. Terms have been defined unequivocally.
- The cuticular characters and their position along the trunk can be plotted unequivocally on a trunk map, fitting with the triangular trunk shape of The Allomalorhagida.
- The new terminology and refined definitions of morphological characters for the different families help to perform precise and homogeneous identifications and descriptions for further taxonomic and phylogenetic studies.
- Around 1500 specimens belonging to 43 species of Allomalorhagida have been identified unequivocally for this Thesis. This includes new species for science, species redescriptions and new reports of known species.
- Eleven new species of Allomalorhagida have been described, one accommodated into the Neocentrophyidae and ten into the Pycnophyidae.
- A new genus and species, *Mixtophyes abyssalis*, collected from the Guinea Basin deep-sea, constitutes the first description of an allomalorhagid genus in 30 years.
- The description of the 11 species included in this Thesis, together with *Pycnophyes norenburgi* (Florida) brings the total number of valid species of Pycnophyidae worldwide from 45 up to 56 (*P.* sp11 from Singapore is not considered because it is currently under revision), an increase of a 20%.

Phylogeny and taxonomical consequences

- New synapomorphic characters for the whole pool of Neocentrophyidae species are recognized.
- Accordingly, new hypothesis on the phylogenetic relationship within the Neocentrophyidae were tested under total evidence analyses.
- Our total evidence analyses strongly support the monophyly of Allomalorhagida.
- Neither *Pycnophyes* nor *Kinorhynchus* were recovered as monophyletic groups. The suggested paraphyly of *Pycnophyes* is verified. *Kinorhynchus* is not valid as a taxonomic and phylogenetic unit and no longer should be considered an allomalorhagid genus.
- The lateral terminal spines (or the rudimental stage of the structure) are a homoplastic character without phylogenetic relevance.
- The internal relationships of Pycnophyidae are now resolved from our total evidence analyses.
- Nine clades supported by morphological and molecular apomorphies and are erected as new genera: *Planolimbus, Cristaphyes, Higginsia, Gymnophyes, Setaphyes, Fujuriphyes, Krakenella,* and *Godzilliphyes.*
- The classification of Pycnophyidae is now inferred from the phylogenetic analysis and not viceversa.
- Neither the topologies yielded by the total evidence analyses nor the character tracing supported the potentially monophyletic group of *Pycnophyes* species without male-specific tubes. Therefore, the absence of the character is consequence of several independent losses.
- Middorsal structure specializations have phylogenetic relevance and their character state evolution was traced through the Allomalorhagida as a transformation series.
- Middorsal spines (plesiomorphic condition for Allomalorhagida), evolved into middorsal spinose processes in Neocentrophyidae and into middorsal elevations in Pycnophyidae, (respectively apomorphic in each family). Within Pycnophyidae, middorsal elevations may be retained, lost or transformed into middorsal processes.
- The species with conspicuous keel-shaped middorsal processes (apomorphy) constitute a fully supported subclade within *Cristaphyes*.
- The presence of lateral terminal spines and the arrangement of segment 1 as a closed ring are the plesiomorphic condition of Allomalorhagida.

Biogeography

- Four of the newly described species were collected from the Iberian Peninsula, constituting the first Pycnophyidae records for the area.
- Four new species are described from the Korean Peninsula, and three from the Guinea Basin deep-sea.
- A total of 15 species of Allomalorhagida are reported along the Iberian coasts, accommodated in four different genera, being the *Pycnophyes* the most diverse so far. The total kinorhynch fauna of the Iberian Peninsula reaches 31 species and 11 genera from 122 sampling localities.
- Apart from the Iberian Peninsula, a total of five species of Allomalorhagida were reported from Italy (Naples), three along the East coast of USA (Maine, Massachusetts, Florida), seven form Panama (Bocas del Toro, Naos), five from Norway (Espegrend), three from Greenland, and one from Singapore.
- In the Iberian Peninsula, most southern Mediterranean localities have a kinorhynch fauna more similar to the Atlantic one, most probably as a result of the strong influence of the Atlantic surface current.
- The results of the present Thesis on the geographic distribution of kinorhynchs can be used as a model for other, non-vagile organisms of the meiofauna.

Morphology

- The organization of cuticular plates on segments 1 and 11 has been updated and reevaluated after the description of the new genus *Mixtophyes* and the re-observation of *Neocentrophyes*.
- Striking cuticular features, such as the keel-shaped middorsal processes, are categorized through careful LM and SEM studies.
- A comprehensive matrix of nearly 100 morphological characters of Allomalorhagida has been compiled for the present Thesis, including their accurate definitions.
- Micro-CT is revealed as a high potential, non-destructive and non-invasive technique, allowing studying both museum specimens and rare taxa with very few available specimens which can be used again with other techniques and purposes.
- Micro-CT techniques give an integrative view of the functional anatomy, making possible to "travel inside" the animal in a 3D rendering. Complex and movable organs, such as the introvert and its intricate associated muscles can be studied “in situ“.

- The optimization of micro-CT techniques to tiny sized animals, such are kinorhynchs, will open a new window to the morphological study of other meiofaunal groups.

CONCLUSIONES

Diversidad y taxonomía

- Se ha establecido una terminología precisa y adecuada para los caracteres morfológicos específicos de los kinorrincos alomalorrágidos. Los términos han sido definidos de manera inequívoca.
- Los caracteres cuticulares y sus posiciones a lo largo del tronco pueden ser situados ahora de forma precisa en un mapa del tronco, de acuerdo con la forma triangular de la sección transversal.
- Tanto la nueva terminología como las definiciones refinadas de los caracteres morfológicos que pueden aparecer en las distintas familias permitirá realizar identificaciones y descripciones precisas y homogéneas en futuros estudios taxonómicos y filogenéticos.
- Se han identificado a nivel de especie aproximadamente 1500 ejemplares distribuidos en a 43 especies de Allomalorhagida; en este cómputo se incluyen tanto las nuevas especies para la ciencia, las nuevas citas de especies ya conocidas y la redescripción de especies.
- Se han descrito once nuevas especies de Allomalorhagida, quedando asignadas una a la familia Neocentrophyidae y diez a la familia Pycnophyidae.
- En nuevo género y especie *Mixtophyes abyssalis*, procedente de la cuenca profunda de Guinea, ha supuesto la primera descripción de un género de alomalorrágidos en 30 años.
- La descripción de las once especies incluidas en esta Tesis, junto con la de *Pycnophyes norenburgi* (Florida), eleva el número total de especies válidas de Pycnophyidae a nivel mundial de 45 a 56 (la especie *P.* sp11 de Singapur no se contabiliza aquí dado que se encuentra en estado de revisión), lo que supone un incremento del 20% en la diversidad de la familia.

Filogenia e implicaciones taxonómicas

- Se han identificado nuevos caracteres sinapomórficos para el conjunto de especies de la familia Neocentrophyidae.
- Se han testado mediante análisis de evidencia total las nuevas hipótesis sobre las relaciones filogenéticas dentro de los Neocentrophyidae.

- Nuestros análisis de evidencia total confirman de manera inequívoca la monofilia de Allomalorhagida.
- Ni *Pycnophyes* ni *Kinorhynchus* se recuperaron como grupos monofiléticos. Así, la hasta ahora supuesta parafilia de *Pycnophyes* queda verificada. *Kinorynchus* no es una unidad válida ni taxonómica ni filogenéticamente y, por lo tanto, no debe considerarse nunca más como un género de alomalorrágidos.
- Las ausencia de espinas lateroterminales (o la presencia del estado rudimentario de dicha estructura) resultó ser homoplásica y sin relevancia filogenética.
- Las relaciones internas de parentesco evolutivo en la familia Pycnophyidae quedan ahora resueltas mediante nuestro análisis de evidencia total.
- Del análisis se obtuvieron nueve clados en la familia Pycnophyidae, todos ellos apoyados por apomorfías morfológicas y moleculares, que se erigieron como nuevos géneros: *Planolimbus*, *Cristaphyes*, *Higginsia*, *Gymnophyes*, *Setaphyes*, *Fujuriphyes*, *Krakenella* y *Godzilliphyes*.
- La clasificación de la familia Pycnophyidae está ahora inferida a partir de un análisis filogenético y no a la inversa.
- Ni las topologías producidas por el análisis de evidencia total ni el rastreo de caracteres confirmaron la potencial monofilia del grupo de especies de *Pycnophyes* sin tubos en los machos. Por lo tanto, la ausencia del carácter en varias ramas terminales se debe a pérdidas producidas de manera independiente.
- Las especializaciones mediodorsales tienen relevancia filogenética y se trazó la evolución de los estados del carácter en los Allomalorhagida.
- Las espinas mediodorsales (condición plesiomórfica para los Allomalorhagida), evolucionaron hacia los salientes espinosos mediodorsales en los Neocentrophyidae y hacia las elevaciones mediodorsales en los Pycnophyidae (caracteres apomórficos de cada familia respectivamente). Dentro de los Pycnophyidae, las elevaciones mediodorsales pueden mantenerse, perderse o bien transformarse en salientes mediodorsales.
- Las especies con salientes mediodorsales prominentes en forma de quilla (apomorfía) forman un subclado fuertemente confirmado dentro del género *Cristaphyes*.
- Tanto la presencia de espinas lateroterminales como la organización del segmento 1 en forma de anillo constituyen la condición plesiomórfica de todos los Allomalorhagida.

Biogeografía

- Se han obtenido cuatro de las especies recientemente descritas en aguas de la Península Ibérica, lo que supone las primeras citas de la familia Pycnophyidae en la zona.
- Se han descrito cuatro nuevas especies de la Península Coreana y tres de la cuenca profunda de Guinea.
- En la Península Ibérica, se han identificado un total de 15 especies de Allomalorhagida, pertenecientes a cuatro géneros distintos, de los que *Pycnophyes* es el más diverso. La fauna total de kinorrincos de la Península Ibérica se eleva a 31 especies repartidas en 11 géneros, recolectadas en 122 localidades de muestreo.
- Además de los datos de la Península Ibérica, se identificaron un total de cinco especies de Allomalorhagida procedentes de Italia (Nápoles), tres de la costa Este de EEUU (Maine, Massachusetts, Florida), siete de Panamá (Bocas del Toro y Naos), cinco de Noruega (Espegrend), tres de Groenlandia y una de Singapur.
- Dentro de los datos de la Península Ibérica, llama la atención que las localidades mediterráneas localizadas más al sur tienen una fauna de kinorrincos más similar a la de las localidades atlánticas, probablemente por la fuerte influencia de la corriente superficial atlántica.
- Los resultados de la presente Tesis Doctoral en cuanto a la distribución geográfica de los kinorrincos pueden ser utilizados como modelo para otros organismos de la meiofauna con escasa capacidad dispersiva.

Morfología

- La organización de las placas cuticulares de los segmentos 1 y 11 ha sido actualizada y reevaluada tras la descripción del nuevo género *Mixtophyes* y la reobservación de los ejemplares tipo de *Neocentrophyes*.
- Los caracteres cuticulares extraños, como los salientes mediodorsales en forma de quilla, se han categorizado mediante exhaustivos estudios con LM y SEM.
- Se ha elaborado una matriz con aproximadamente 100 caracteres morfológicos de Allomalorhagida, para cada uno de los cuales se han redactado definiciones precisas.
- El Micro-CT se ha revelado cono una técnica de gran potencial, no destructiva y no invasiva, que permite el estudio tanto de ejemplares de museo como de taxones raros con baja disponibilidad de individuos. Tras su estudio, los ejemplares pueden ser reutilizados con diferentes fines mediante otras técnicas.

- Las técnicas del Micro-CT dan una visión integradora de la anatomía funcional, haciendo posible “viajar” dentro del animal en las 3 dimensiones del espacio. Los órganos complejos y móviles, como el introverto y su compleja musculatura asociada, pueden ser así estudiados *in situ*.
- La optimización de las técnicas de Micro-CT para animales de tan reducido tamaño, como es el caso de los kinorrincos, abre una nueva ventana a los estudios morfológicos de otros grupos de la meiofauna.

6

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